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Presidential Plenary

PL-1

LDV/SOF + RBV in HCV patients with decompensated cirrhosis or liver transplantation: SOLAR-1 + 2

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Background: Treatment options for patients with hepatitis C virus (HCV) who have decompensated cirrhosis or who have undergone liver transplantation are limited.

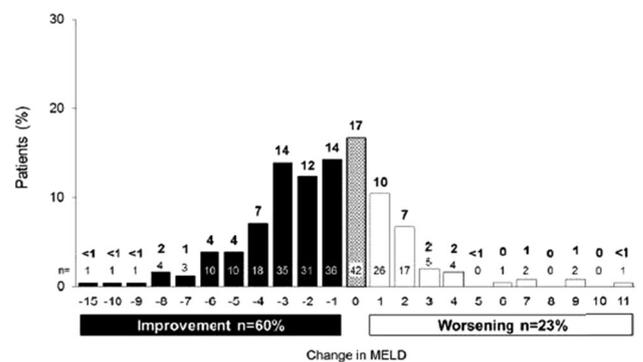
Methods: Data from the SOLAR-1 and SOLAR-2 studies, in which patients with HCV genotype(GT) 1 or 4 were randomized to receive 12

or 24 weeks of ledipasvir (LDV)/sofosbuvir (SOF) + ribavirin(RBV): patients without a transplant with (1) Child-Pugh-Turcotte (CPT) B or (2) CPT-C cirrhosis; or transplanted patients with (3) no cirrhosis (F0 to F3), (4) CPT-A, (5) CPT-B or, (6) CPT-C cirrhosis, or (7) fibrosing cholestatic hepatitis. We evaluated, SVR12, relapse and change from baseline in CPT and MELD scores 12 weeks after the end-of-treatment among those patients with SVR12.

Results: Of 667 patients analyzed, 27 have not reached post-treatment week 12 visit. Overall, 575/627 (92 %) subjects achieved SVR12; 545/590 (92 %) and 30/37 (81 %) in GT1 and GT4 infection, respectively. Relapse rates were low; 23/598 (4 %) overall, 20/565 (4 %) and 3/33 (9 %) in GT1 and GT4 infection, respectively. Relapse occurred more commonly in decompensated patients, but was not related to treatment duration. Of the 250 decompensated patients who achieved SVR12, 60 % (150) had an improvement in MELD scores (see Table) from baseline to post-treatment week 12, 61 % (41/67) with baseline MELD \geq 15, had a post-treatment week 12 MELD of $<$ 15, and 66 % (164/248) had improvement in CPT scores.

Conclusions: LDV/SOF + RBV for 12 or 24 weeks in patients with decompensated cirrhosis or recurrent HCV was efficacious with low relapse rates. Most patients with SVR12 also had improvements in CPT and MELD scores.

Changes in MELD scores from baseline to post-treatment week 12



PL-2

Sofosbuvir/velpatasvir for the treatment of HCV decompensated liver disease patients: ASTRAL-4 study

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Introduction: Velpatasvir (VEL) has demonstrated high SVR rates in patients with genotypes 1–6 HCV when used in combination with sofosbuvir (SOF). This Phase-3 study evaluated the safety and efficacy of the fixed-dose-combination (FDC) of SOF/VEL in HCV-infected patients with decompensated liver disease.

Methods: Genotype (GT) 1, 2, 3, 4 or 6 HCV infected patients with CPT-B cirrhosis were randomized 1:1:1 to receive SOF/VEL (400 mg/100 mg) daily for 12-weeks, SOF/VEL + weight-based RBV for 12-weeks, or SOF/VEL for 24-weeks. Patients with prior liver transplant or hepatocellular carcinoma were excluded.

Results: Of the 267 patients treated, most were male (70 %), white (90 %) and treatment experienced (55 %). Patients had genotype 1 (78 %), 2 (4.5 %), 3 (15 %), 4 (3 %) or 6 (<1 %) HCV infection. The SVR12 rates are shown in Table 1. SOF/VEL + RBV for 12 weeks resulted in high SVR rates with virologic failure occurring in 1 (1 %) of GT-1 and 2 (15.2 %) of GT3 subjects respectively, including virologic breakthrough in a single GT3 patient. Among patients who achieved SVR, 47 and 56 % had improvements in CPT and MELD scores respectively. The most common adverse events were fatigue, headache, nausea. Overall 9 patients discontinued SOF/VEL due to adverse events. 47(18 %) patients experienced serious adverse events and there were 9 deaths; none were related to study drug.

Conclusions: SOF/VEL + RBV for 12-weeks resulted in high SVR rates across all HCV genotypes in decompensated patients with early improvements in liver function. This regimen was well tolerated with AEs consistent with clinical sequelae of advanced liver disease and RBV.

Table 1. Virologic Outcomes By Genotype

	Total (All GTs)	GT-1	GT-2	GT-3	GT-4	GT-6
SOF/VEL 12 Week Group						
SVR12	75/90 (83.3%)	60/68 (88.2%)	4/4 (100.0%)	7/14 (50.0%)	4/4 (100.0%)	NA
SOF/VEL+RBV 12 Week Group						
SVR12	82/87 (94.3%)	65/68 (95.6%)	4/4 (100.0%)	11/13 (84.6%)	2/2 (100.0%)	NA
SOF/VEL 24 Week Group						
SVR12	77/90 (85.6%)	65/71 (91.5%)	3/4 (75.0%)	6/12 (50.0%)	2/2 (100.0%)	1/1 (100.0%)

PL-3

Serum HBV-RNA levels decline significantly in chronic hepatitis B patients dosed with REP2139-CA

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Background and aims: The HBsAg release inhibitor REP2139-Ca may be a promising new treatment option for chronic hepatitis B (CHB) patients, however its effect on hepatitis B pregenomic RNA (HBV-RNA) is unknown. HBV-RNA levels during treatment with REP2139-Ca were determined and compared with HBV-DNA and HBsAg levels.

Methods: 12 Patients with HBeAg positive CHB participating in a phase 2 study were dosed with the nucleic-acid based amphipathic polymer REP2139-Ca for 20–38 weeks. Responders to REP2139 (defined as clearance of serum HBsAg) were subsequently treated with an add-on immunomodulatory agent (peginterferon alpha-2a and/or thymosin alpha-1). HBsAg, HBV-DNA, and HBV-RNA levels were determined at baseline, after 20–24 weeks of REP2139-Ca monotherapy, and either during a treatment-free follow-up (for responders) or during entecavir treatment (for non-responders). HBV-RNA was quantified by RT-qPCR using HBV-specific primers.

Results: HBV-RNA levels were detectable in all 12 HBeAg-positive patients before treatment [mean 6.70 (SD 0.83) logC/mL], and were significantly associated with HBsAg (r^2 0.33, $p = 0.049$) and HBV-DNA levels (r^2 0.74, $p < 0.001$). After 20–24 weeks of REP2139-Ca treatment, mean HBV-RNA, HBV-DNA, and HBsAg levels had declined significantly compared to baseline (all $p < 0.001$). At week 20–24, HBV-RNA was undetectable in 8/12 patients. In 7 of these 8 patients, HBV-RNA remained undetectable during the treatment-free follow-up period (mean 21.9 weeks, range 7–27). HBsAg sero-conversion was achieved in 4/8 patients during follow-up (anti-HBs range 200–766 U/L).

Conclusions: In patients treated with REP2139-Ca, serum HBV-RNA levels declined significantly compared to baseline. REP2139-Ca may be a promising new treatment option for CHB patients.

PL-4

192 weeks tenofovir disoproxil fumarate monotherapy in Chinese patients with chronic hepatitis B

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Aims: Tenofovir disoproxil fumarate (TDF) has demonstrated short-term efficacy and a high resistance barrier in chronic hepatitis B (CHB) populations in China. This Phase III study provides long-term data on TDF treatment in Chinese CHB patients.

Methods: This was an open-label study period, involving TDF monotherapy following a 48-week double-blind randomized treatment period of either TDF 300 mg QD or Adefovir Dipivoxil (ADV) 10 mg QD. HBeAg-positive and negative subjects with HBV DNA $\geq 10^5$ copies/mL were eligible for initial randomization. Totally 497/512 (97 %) subjects (198 HBeAg positive and 299 HBeAg negative) entered the open-label phase; 252 subjects originally randomized to TDF (TDF-TDF) and 245 subjects randomized to ADV (ADV-TDF). The majority of subjects (95.5 %) were treatment naïve. Virologic, serologic, biochemical, safety and resistance were monitored throughout the study.

Results: At Week 192, virologic suppression (HBV DNA <400 copies/mL) was achieved in the majority of treated subjects (TDF-TDF Vs. ADV-TDF) in HBeAg positive (91.3 vs. 92.9 %, $p > 0.05$) and HBeAg negative (92.9 vs. 92.2 %, $p > 0.05$). More than 80 % subjects achieved ALT normalization. A higher proportion of subjects in the TDF-TDF group experienced HBeAg loss and HBeAg seroconversion but these differences were not statistically significant. No subject experienced durable HBsAg loss/seroconversion. No TDF resistance mutations were identified. More than 92 % subjects completed 192-week treatment and TDF long-term safety profile was as previously established.

Conclusions: TDF demonstrated high potency, no resistance, and good tolerability in Chinese CHB subjects receiving 192 week TDF monotherapy.

PL-5

Juntendo University radiofrequency ablation (RFA) training program

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RFA is a standard treatment modality for liver tumors. However, RFA is highly operator-dependent. Its skills and outcomes are different from operator to operator, or from institution to institution. Our institution is the highest volume center of RFA in Japan now. To

disseminate skills and know-hows for RFA, we held RFA training programs six times and 99 doctors participated in. The programs were popular and the quota was filled every time. The 2-day program was composed of comprehensive lectures, live demonstrations and case studies. Content of the lectures were current status of RFA, RFA devices, ultrasonography, and others. The live demonstration included presentation of three cases on which we would have performed RFA on the next day, and planning ultrasound examinations conducted by participants. On the second day, we performed RFA on the three cases, using artificial ascites technique, contrast-enhanced ultrasound guidance, and fusion imaging. In the case studies, around 12 difficult to ablate cases from participants' hospitals were presented and discussed. Through the intensive program, participants learned the current status of RFA. A questionnaire survey after the program revealed overwhelmingly positive feedback from the participants. Many participants remarked on the benefit of being directly trained by the noted interventional oncologists in an academic environment where interaction between teachers and students and group discussions were encouraged. In the future, we would also hold a longer-term training program for such as 2 weeks. Additionally, we would have an international training program for foreign doctors.

PL-6

AARC-ACLF Score predicts 30 day survival better than CLIF-SOFA and MELD scores in patients with ACLF

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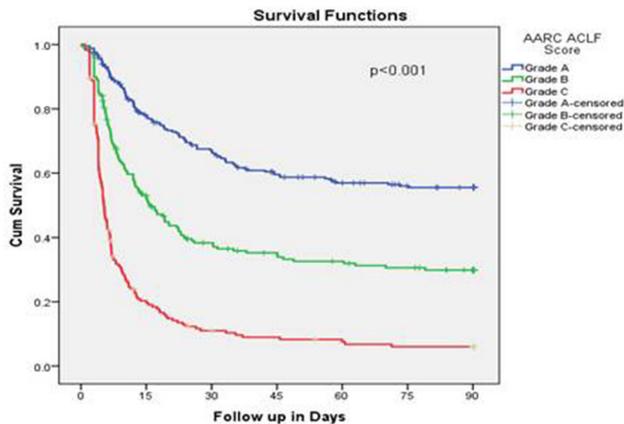
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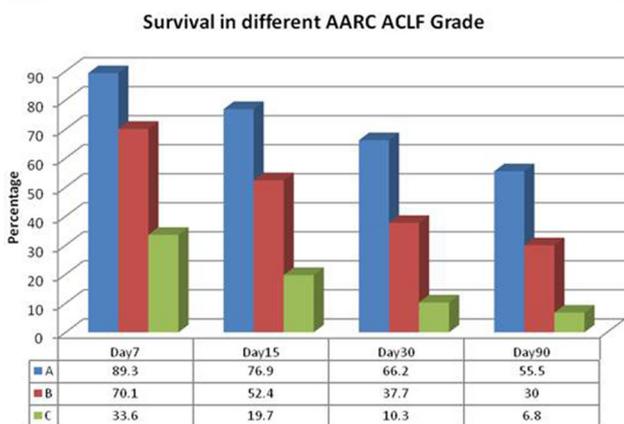
Background and aims: Acute on chronic liver failure (ACLF) is associated with the rapid worsening of liver failure with high mortality. Prediction of survival and early intervention can improve the outcome. Aim was to derive a prognostic model in patients of ACLF by APASL definition.

Fig-1: Survival at time point in different AARC ACLF Grade



Methods: Total 1021 ACLF cases with 90 days follow up enrolled into the APASL ACLF Research Consortium (AARC) were analyzed. A derivation set of 338 cases analyzed for a prognostic model and calibrated in 683 cases validation set.

Fig-2: Survival at time points –AARC ACLF Grade wise.



Results: Of all the baseline independent predictor of mortality, total bilirubin, Creatinine, Lactate, INR and hepatic encephalopathy were considered. AUROC in derivation and validation cohort were 0.797 and 0.793 respectively. AARC ACLF score was developed with a minimum and maximum of 5 and 15. The score was better than the MELD and CLIF SOFA with an AUROC of 0.76, sensitivity 70 %, specificity 67 %, PPV 78 % and NPV of 58 % in predicting 90 days survival. Grading was done with Grade A (5–9), Grade B (10–11) and Grade C (12–15 points). The mortality risk increases by 9.7 % with each unit increase. Score of 11 at baseline or persistence of the same in first week associated with 100 % mortality in 30 days. Overall median survival was 26.3 days and that of Grade B, C being 16 and 5 days respectively and overall survival of 51.8 %.

Conclusion: The AARC ACLF score is dynamic, simple and better to the existing models. The definitive therapies i.e. transplant can be predicted within first week.

Table-1: AARC ACLF Score

Points	Bilirubin (mg/dl)	HE Grade	INR	Lactate (mmol/lit)	Creatinine (mg/dl)
1	<15	0	<1.5	<1.5	<1.0
2	15.01-22	I-II	1.5-2.5	1.5-2.5	1.01-1.5
3	>22	III-IV	>2.5	>2.5	>1.5

Table-2: AARC ACLF Grade

Grade	Score
A	5-9
B	10-11
C	12-15

Table 3: Comparison with CLIF SOFA, MELD, AARC ACLF Score without creatinine

Score	AUROC	Cutoff	Sensitivity	Specificity	Positive Predictive Value	Negative Predictive Value
AARC Score	0.76	9.5	70.4	66.8	77.9	57.6
MELD	0.74	30	69.6	69.7	80.0	57.4
AARC Score without creatinine	0.73	7	79.0	53.0	73.6	60.3
CLIF-SOFA	0.70	10.5	72.7	55.5	72.1	56.2

Oral Presentation

O-001

Analysis of cellular factors involved in the particle formation and secretion of hepatitis C virus

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Background: Despite the development of JFH-1 based HCVcc systems in 2005, the mechanism of virus particle formation and assembly of HCV is not still well-understood. We identified that Glutathione peroxidase 8 (GPx8), a membrane-associated peroxidase involved in disulfide bond formation in the endoplasmic reticulum, as a novel cellular substrate of the HCV NS3-4A protease. The aim of this study was to analyze the association between GPx8 and HCV life cycle, especially focused on HCV particle formation and assembly.

Material & methods: We generated the plasmid harboring the GPx8 and its variants. These GPx8 and variants plasmids are inserted using gene mutation of siRNA-resistant, so these clones allow us to exclude the influence of endogenous GPx8. We analyzed the GPx8 and variants overexpression and knockdown cell lines with HCVpp and HCVcc whether GPx8 affects HCV life cycle.

Results: The functional studies of GPx8 on HCV life cycle, involving overexpression and RNA silencing, revealed that GPx8 is a proviral factor involved in viral particle production but not in HCV entry or RNA replication.

Conclusions: GPx8 is a proviral host factor cleaved by the HCV NS3-4A protease. Studies investigating the consequences of cleavage for GPx8 function are underway. The identification of novel cellular substrates of the HCV NS3-4A protease should yield new insights into the HCV life cycle and the pathogenesis of hepatitis C and may reveal novel angles for therapeutic intervention.

O-002

Shortened 12-week simeprevir/peginterferon/ribavirin therapy for GT 1b chronic hepatitis C patients

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Background: Although interferon-free regimens are becoming popular in the treatment of chronic hepatitis C, resistance to direct-acting antiviral agents is an obstacle to viral eradication. Interferon-based therapy is less likely to induce resistance and is, more importantly, cheaper than interferon-free regimens. We aimed to evaluate the efficacy of 12-week simeprevir/peginterferon/ribavirin treatment in genotype 1b chronic hepatitis patients who were untreated or relapsed.

Methods: This is an open-label single arm multicenter study (study ID: UMIN000012661). Patients received 12-week simeprevir (100 mg)/peginterferon alfa-2a (180 µg)/ribavirin treatment. If serum HCV-RNA was undetectable at weeks 2, 4 and 8, treatment was stopped at week 12. The other patients received peginterferon/ribavirin treatment for additional 12 weeks (totally 24 weeks).

Results: Ninety patients were enrolled. Thirty patients (35 %) negative for HCV-RNA at week 2 received 12-week treatment (12-week group). Those positive for HCV-RNA at week 2 received 24-week treatment (24-week group). SVR was achieved in 71 and 79 % of 12- and 24-week group, respectively. IL-28B genotype (rs8099917) was

the only significant predictor for achieving SVR. All of the IL-28B TT patients (16 and 27 patients in 12- and 24-week group, respectively) achieved SVR. In contrast, SVR rate of IL-28B non-TT patients was 42 and 52 % in 12- and 24-week group, respectively. In conclusion, all IL-28B TT patients negative for HCV-RNA at week 2 achieved SVR by 12-week treatment. Therefore, simeprevir/peginterferon/ribavirin therapy is an option in the treatment of IL-28B TT patients, and treatment duration could be shortened to 12 weeks for those with very rapid virologic response.

O-003

Treatment with anti-MiRNA122 RG-101 results in decreased IP-10 in patients with chronic hepatitis C

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Aim: MicroRNA-122 is an important host factor for hepatitis C virus replication. RG-101, an oligonucleotide targeting miR-122, may be a new treatment option for patients with chronic hepatitis C (CHC). Oligonucleotides can potentially trigger innate immune sensors. Here, we have analyzed immunological responses in the plasma of patients with CHC who were dosed with RG-101.

Methods: 32 Patients with CHC participated in a phase 1 study and received a single subcutaneous injection with RG-101. 14 patients received 2 mg/kg, 14 patients 4 mg/kg, and 2 patients in each group received placebo. Cytokine and chemokine levels were measured in all patients and 6 healthy controls using a Luminex 20-plex immunoassay (Affymetrix eBioscience, CA, USA) at baseline, day 3, 8, 29 and 57.

Results: After dosing with RG-101 IP-10 levels had declined significantly as compared to placebo (1.12 fold reduction, day 8) in both the 2 mg/kg (2.13 fold reduction, $p = 0.0046$) and 4 mg/kg group (2.56 fold reduction, $p = 0.0044$). The decrease in IP-10 was independent of HCV RNA decline (Pearson $r = 0.27$, $p = 0.15$) and decrease in IP-10 was similar between patients with and without HCV RNA below the limit of quantification at day 57 (2.38 and 2.17 fold reduction respectively, $p = 0.64$). Other cytokines and chemokines measured showed no differences between healthy controls and patients with CHC, and no differences in CHC patients after dosing with RG-101.

Conclusions: RG-101, does not trigger systemic immune activation. In CHC patients dosed with RG-101, a significant decline was observed in IP-10 serum concentrations compared to baseline.

O-004

Impact of the universal drug coverage program to eradicate the burden of hepatitis C in Portugal

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Aims: A survey by the Portuguese Ministry of Health (PMH) in 2014 identified 13,015 hepatitis C patients in need for treatment with new generation direct-acting antivirals. In February 2015 the PMH initiated a new hepatitis C treatment policy granting universal access to sofosbuvir and ledipasvir/sofosbuvir. Long-term impact of the first 6 months of policy implementation is reported.

Methods: Lifetime impact of hepatitis C virus (HCV) infection and treatment outcomes were estimated using a discrete-time Markov. Impact of the new policy relative to the alternatives before policy implementation was measured in terms of mortality (life expectancy, HCV-related deaths) and morbidity (cases potentially averted; decompensated cirrhosis-DC, hepatocellular carcinoma-HCC, liver transplants-LT) and related treatment costs (euros). Overall survival was measured with the Kaplan-Meier estimator.

Results: After 6 months 6253 patients were included in the new policy: mean (SD) age 51.7 (10.3) years; 73.5 % male; HCV G1 (69.5 %) G2 (1.2 %) G3 (17.0 %) G4 (12.2 %) G5–6 (0.1 %); METAVIR F0 (2.4 %) F1 (17.2 %) F2 (17.7 %) F3 (21.8 %) F4 (40.8 %); HIV-positive (27.2 %); HCV treatment-experienced (48.5 %). Treatments authorized were: ledipasvir/sofosbuvir 12 weeks (n = 2886; 46.2 %); ledipasvir/sofosbuvir 24 weeks (n = 2409; 38.5 %); sofosbuvir 12 weeks (n = 169; 2.7 %); sofosbuvir 24 weeks (n = 523; 8.4 %); others (n = 266; 4.2 %). Less 2634 HCV-related deaths are estimated to result in 7.7 years/patient life expectancy increment and 47,839 life years gained. A total of 2170 cases of DC and 1455 HCC and 260 LT cases can be averted while decreasing 201.8 million euros to public expenditure.

Conclusions: Unprecedented gains in patients' life expectancy and substantial reduction in the costs of treating hepatitis C consequences are expected with the Portuguese universal access policy to new generation direct-acting antivirals.

O-005

Safety and efficacy of hepatitis C genotype 1b oral regimens: HCV-target interim analysis

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Background and aims: The aim of this study is to evaluate the safety and efficacy of all oral regimens for the treatment of patients with genotype 1b (G1b) in HCV-TARGET, a multicentre, prospective, observational cohort study.

Methods: Patients who initiated HCV treatment in clinical practice were enrolled and treated according to the regional standards of care at academic (n = 40) and community medical centres (n = 17) in North America (n = 53) and Europe (n = 4). Information was

collected from the medical records and abstracted into a unique centralized data core. Demographic, clinical, adverse events (AEs) and virological data are collected throughout treatment and follow-up. **Results:** To date, 1218 G1b pts have initiated an all-oral regimen; which include Sofosbuvir(SOF)/Ledipasvir(LDV) ± RBV (n = 614), SOF + Simprevir(SMV) ± RBV (n = 344), paritaprevir/R/ombitasvir/dasabuvir ± RBV (n = 201), and Daclatasvir(DCV) + SOF ± RBV (n = 23). Demographics include 58 % male, mean age of 61, 19 % Black, 44 % cirrhosis (19 % decompensation Hx), and 12 % with liver transplant. 53 % of pts had received prior HCV therapy, with 1/3 of these having failed a prior DAA-based regimen. A per protocol analysis shows SVR12 of: SOF/LDV ± RBV 98 % (292/299), SOF + SMV ± RBV 93 % (276/297), paritaprevir/R/ombitasvir/dasabuvir ± RBV 97 % (33/34), DCV + SOF ± RBV 93 % (14/15). For the entire cohort, 16 patients have discontinued treatment early due to AEs and there were 4 deaths (SOF/LDV n = 3, DCV + SOF ± RBV n = 1). Complete safety and efficacy data for the cohort will be presented.

Conclusions: Preliminary safety and efficacy data from HCV-TARGET for patients with G1b HCV suggests that all-oral treatment regimens are generally safe and highly efficacious across a broad spectrum of patients including cirrhotics.

O-006

GIFT-I: durability of response of ombitasvir/paritaprevir/ritonavir in HCV GT1b Japanese patients

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Background: To evaluate the durability of response of an all-oral, IFN/RBV-free regimen of coformulated ombitasvir/paritaprevir/r (OBV/PTV/r 25/150/100 mg QD [PTV identified by AbbVie and Enanta]) in HCV genotype 1b-infected Japanese patients without cirrhosis or with compensated cirrhosis.

Methods: Non-cirrhotic treatment-naïve and IFN-experienced Japanese patients with HCV GT1b infection were randomized 2:1 to receive double-blind (DB) OBV/PTV/r for 12 weeks (Arm A) or DB placebo for 12 weeks followed by open-label (OL) OBV/PTV/r for 12 weeks (Arm B). Patients with compensated cirrhosis received 12 weeks of OL OBV/PTV/r (Arm C). This analysis assessed sustained virologic response (HCV RNA < LLOQ [<25 IU/mL]) at post-treatment week 24 (SVR24). Treatment-emergent adverse events (TEAE) from first dose through 30 days post-dose are presented for patients who received ≥ 1 dose of active study drugs. Post-treatment week 48 data will be available at the time of the meeting.

Results: 215, 106, and 42 patients were treated in Arms A, B, and C, respectively. All patients who achieved SVR12 also achieved SVR24, except one patient in Arm A who relapsed after post-treatment week 12 (Table). Most TEAEs were grade 1 or 2 in severity. The most common TEAEs were nasopharyngitis (13.7 %) and headache (8.1 %) in non-cirrhotic patients and nasopharyngitis (14.3 %) in cirrhotic patients. Serious TEAEs occurred in 7, 3, and 2 patients in Arms A, B, and C, respectively.

Conclusions: Among 363 patients treated in this study, concordance between SVR12 and SVR24 was 99.7 % (362/363 patients). Of 344 patients who achieved SVR12, only 1 patient relapsed by SVR24.

	Arm A DB OBV/PTV/r Non-cirrhotic N=215	Arm B OL OBV/PTV/r Non-cirrhotic N=106	Arm C OL OBV/PTV/r Compensated Cirrhosis N=42
SVR12, n/N (%)			
Overall	204/215 (94.9)	104/106 (98.1)	38/42 (90.5)
Treatment-naïve	131/139 (94.2)	67/68 (98.5)	N/A
IFN-experienced	73/76 (96.1)	37/38 (97.4)	N/A
SVR24, n/N (%)			
Overall	203/215 (94.4)	104/106 (98.1)	38/42 (90.5)
Treatment-naïve	130/139 (93.5)	67/68 (98.5)	N/A
IFN-experienced	73/76 (96.1)	37/38 (97.4)	N/A
Reasons for not achieving SVR24 n/N (%)			
Non-response	12/215 (5.6)	2/106 (1.9)	4/42 (9.5)
OTVF	1/215 (0.5)	1/106 (0.9)	1/42 (2.4)
Relapse by PTW12	5/209 (2.4)	1/105 (1.0)	2/40 (5.0)
Relapse between PTW12 and PTW24	1/204 (0.5)	0/104	0/38
SD discontinuation	5/215 (2.3)	0/106	0/42

DB:double-blind; N/A: not available; OBV/PTV/r:ombitasvir/paritaprevir/r 25/150/100 mg QD, 12 weeks; OL:open-label; OTVF:on-treatment virologic failure; PTW:post-treatment week; SD:study drug.

O-007

UNITY-3: DCV/ASV/BCV in treatment-naïve and IFN-experienced Japanese patients with HCV GT1 infection

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Background: The DCV-TRIO regimen is the all-oral, ribavirin-free, fixed-dose combination of daclatasvir 30 mg (pangenotypic NS5A inhibitor), asunaprevir 200 mg (NS3 protease inhibitor), and beclabuvir 75 mg (non-nucleoside NS5B inhibitor). The efficacy and safety of DCV-TRIO was studied in Japanese patients with chronic HCV genotype (GT) 1 infection; DCV-TRIO has achieved overall SVR12 rates ≥ 96 % in patients with GT1b infection in previous global studies.

Methods: Treatment-naïve, GT1b-infected Japanese patients were randomly assigned to receive blinded DCV-TRIO for 12 weeks or DUAL for 24 weeks. Interferon-experienced and GT1a-infected patients received open-label DCV-TRIO for 12 weeks.

Results: Patients who received DCV-TRIO had a median age of 64 years (range 27–80); 21 % had compensated cirrhosis. SVR12 rates ≥ 95 % were achieved in both treatment-naïve (GT1b, n = 149; GT1a, n = 3) and -experienced (GT1b, n = 64; GT1a, n = 1) cohorts treated with DCV-TRIO (Table). 87 % of DUAL recipients (N = 75) achieved SVR12, including 58/59 (98 %) patients without baseline NS5A polymorphisms (L31 or Y93H). Among GT1b

patients who completed ≥ 4 weeks of DCV-TRIO, 100 % of those with posttreatment data achieved SVR12 irrespective of baseline L31 or Y93H polymorphisms. Adverse events (AEs) led to treatment discontinuation in 10 % of DCV-TRIO recipients, mostly due to liver-related events. Treatment-related serious AEs occurred in 4 % of DCV-TRIO recipients. Seven patients (9 %) discontinued DUAL due to AEs. There were no deaths.

Conclusion: High SVR12 rates (≥ 95 %) were achieved after 12 weeks of treatment with the DCV-TRIO regimen in Japanese patients with GT1 infection, including those with cirrhosis. DCV-TRIO had an overall tolerable safety profile that was comparable with DUAL.

	DCV-TRIO (N=217)	DCV-DUAL (N=75)
End of treatment response, ^a n/N (%)	206/217 (95)	71/75 (95)
SVR12, ^b n/N (%)	208/217 (96)	65/75 (87)
Naïve	146/152 (96) ^c	65/75 (87)
Experienced	62/65 (95) ^d	NA
Cirrhosis	44/46 (96)	13/14 (93)
No Cirrhosis	164/171 (96)	52/61 (85)
Serious AEs, n (%)	13 (6)	8 (11)
Treatment-related	8 (4)	2 (3)
Discontinuations due to AEs, n (%)	21 (10)	7 (9)

^a HCV RNA undetectable (below lower limit of quantitation, target not detected) at the end of treatment; ^b HCV RNA below lower limit of quantitation, target detected or target not detected, at posttreatment Week 12; ^c Includes 3 patients with GT1a infection who achieved SVR12; ^d Includes 1 patient with GT1a infection who relapsed; NA, not applicable.

O-008

All-oral daclatasvir/asunaprevir/beclabuvir fixed-dose combination therapy for HCV genotype 1

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Background: The DCV-TRIO regimen is an all-oral, ribavirin-free, fixed-dose combination of daclatasvir 30 mg (pangenotypic NS5A inhibitor), asunaprevir 200 mg (NS3 protease inhibitor), and beclabuvir 75 mg (non-nucleoside NS5B inhibitor). We assessed the efficacy and safety of DCV-TRIO in patients with chronic HCV genotype (GT) 1 infection in a phase 3 study conducted in Korea, Taiwan and Russia.

Methods: Patients received DCV-TRIO twice daily for 12 weeks. SVR12 rates and safety in treatment-naïve (N = 138) and -experienced (N = 31) cohorts were evaluated separately as key outcomes.

Results: Overall, at baseline, 23 (14 %) patients had compensated cirrhosis, 88 (52 %) were male, 81 (48 %) Taiwanese, 78 (46 %) Korean and 52 (31 %) IL28B (rs1297860) non-CC genotype. NS5A resistance-associated variants (RAVs) were detected at baseline in 25/165 (15 %) patients with available GT 1 NS5A sequence data. SVR12 was achieved by 99 % of treatment-naïve and 100 % of treatment-experienced patients (Table). The two treatment-naïve patients with relapse were subsequently found (by sequence alignment) to be infected with HCV GT 6 g; overall, 100 % SVR12 was observed in HCV GT 1a-infected (N = 10) and GT 1b-infected (N = 157) patients. Two patients reported serious adverse events. Grade 3/4 ALT elevations were reported in 7 (4 %) patients and led to discontinuation in 4 (2 %) patients; all achieved SVR12. There were no Grade 3/4 total bilirubin increases and no deaths.

Conclusion: Twelve weeks of DCV-TRIO treatment was well tolerated and resulted in 100 % SVR12 rates in treatment naïve and IFN experienced patients with HCV GT 1 infection, with or without cirrhosis, including those with baseline NS5A RAVs.

	Treatment-naïve DCV TRIO (N=138)	Treatment-experienced DCV TRIO (N=31)
Virologic outcomes, n (%)		
SVR12	136 (98.6)	31 (100%)
Relapse	2 (1.4)*	0
On-treatment failure	0	0
Safety outcomes, n (%)		
Serious adverse events	2 (1.4) [†]	0
Adverse events leading to discontinuation	2 (1.4)	2 (6.5)

*Sequence alignment analysis showed that the 2 patients with relapse were infected with HCV GT 6g.
[†]Syncope (related) and URTI; fractured patella.

O-009

An Integrated analysis of phase 2,3 SOF and LDV/SOF trials for the treatment of GT6 HCV infection

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Introduction: Chronic HCV infection is endemic in South East Asia with HCV genotype 6 (GT6) accounting for 18–49 % of those infected. Few studies have examined the efficacy and safety of direct acting antiviral (DAA) regimens in GT6 infected patients. The aim of this integrated analysis was to characterize the efficacy and safety of sofosbuvir (SOF)-based regimens in patients with chronic GT6 HCV infection.

Methods: GT6 infected subjects were identified in 5 studies (ATOMIC, NEUTRINO, GS-US-334-0115, ELECTRON2, GS-US-337-0131) and are included in this analysis. Treatment-naïve or treatment-experienced patients received SOF + RBV ± Peg-IFNα or ledipasvir (LDV)/SOF for 12–24 weeks. The primary efficacy endpoint in all studies was SVR12.

Results: A total of 52 subjects with GT6 HCV infection were identified. The majority were treatment-naïve (94 %), Asian (81 %), male (58 %), and had IL28B CC alleles (81 %). The mean age was 50 years (range 26–76) and 10 % had cirrhosis. GT6 subtypes included 6a, 6a/b, 6c-1, 6e, 6 g, 6j, 6 l, 6 m, 6o, 6p, 6q, and 6r. One subject in ELECTRON2 withdrew consent after receiving 8 weeks of LDV/SOF and relapsed with the emergent NS5B RAV S282T. All remaining 51 patients achieved SVR12, including 100 % (3/3) experienced and 100 % (5/5) cirrhotics. SVR12/24 results will be presented.

Conclusions: SOF + RBV ± Peg-IFN α and LDV/SOF regimens are well-tolerated and highly effective in patients with chronic GT6 HCV infection including those who are treatment experienced and have compensated cirrhosis. These regimens provide multiple therapeutic options for consideration when evaluating optimal therapy for individual patients with chronic GT6 HCV infection.

SVR12 Rates in GT6 HCV Patients	SOF + RBV	SOF + RBV	LDV/SOF	Overall (n=52)
	12 or 24 weeks ¹ (n=11)	12, 16, or 24 weeks ² (n=11)	12 weeks ³ (n=30)	
SVR12, % (n/N)	100% (11/11)	100% (11/11)	97% (29/30)	98% (51/52)

¹ATOMIC (P3977-074); NEUTRINO (GS-US-334-0115); GS-US-334-0115; ELECTRON2 (GS-US-337-0131); GS-US-337-0131

O-010

Asian patients with GT 2 HCV achieve 98 % SVR with 12 weeks of sofosbuvir & ribavirin

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Introduction: Chronic hepatitis C (CHC) infection with HCV genotype (GT)2 represents a significant burden on public health in Asia. Sofosbuvir (SOF) in combination with ribavirin (RBV) is the first all-oral regimen for treatment of HCV GT2 infection. The aim of this integrated analysis is to characterize the efficacy and safety of SOF + RBV in a large cohort of Asian patients with HCV GT2 infection from two Phase-3 trials, GS-US-334-0118 (Japan) and GS-US-334-0115 (Korea and Taiwan).

Methods: In both studies, adults with chronic GT2 HCV infection received SOF (400 mg) combined with RBV for 12 weeks. The

primary efficacy endpoint was Sustained Virologic Response measured 12 weeks after the last dose of study drug (SVR12).

Results: Overall, 369 patients were enrolled (n = 129 Korea, n = 87 Taiwan, and n = 153 Japan). Mean age (range) was 55 (22–82) years, 44 % male, and 12 % had cirrhosis. The overall SVR12 rate was 98 % (360/369). Similar high SVR12 rates were seen in treatment-experienced (98 %), patients ≥ 65 years old (96 %), and those with cirrhosis (98 %). Nine patients did not achieve SVR12 (1 partial responder, 6 relapsers, 2 lost to follow up). Adverse events were generally mild to moderate. Laboratory abnormalities were consistent with the safety profile of RBV. No AEs led to treatment discontinuation. SVR24 will be presented.

Conclusions: Asian patients with chronic GT2 HCV infection achieved high rates of SVR12 with 12-weeks of SOF + RBV. The regimen was safe and well-tolerated with no treatment discontinuations due to AE. SOF + RBV offers an improved, IFN-free treatment for Asian patients with chronic GT2 HCV infection.

O-011

Ledipasvir/sofosbuvir regimens for the retreatment of patients who failed sofosbuvir-based regimens

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Background: Ledipasvir/sofosbuvir (LDV/SOF) fixed-dose combination tablet is approved for the treatment of genotype-1 HCV-infected patients. This study evaluated the efficacy and safety of LDV/SOF \pm RBV for 12 or 24 weeks in patients who failed prior SOF-based therapies, including those with advanced liver disease. **Methods:** This open-label retreatment study enrolled HCV genotype-1 infected patients who participated in prior SOF or LDV/SOF clinical studies and failed to achieve SVR. NS5A and NS5B resistance-associated variants (RAV) were evaluated by deep sequencing at baseline and virologic failure. The primary endpoint was SVR12.

Results: 100 patients were enrolled: 41 were NS5A-experienced (LDV/SOF \pm RBV failures) and 59 had failed prior treatment with SOF \pm Peg-IFN \pm RBV. Table 1 presents the baseline characteristics and virologic response by LDV/SOF retreatment regimen. Overall, the mean age was 57 years, 72 % were male, 81 % Caucasian, 93 % IL28B CT / TT, 67 % genotype-1a, 41 % with cirrhosis, and mean baseline HCV RNA was 6.2 log₁₀ IU/mL. At baseline, 37 patients had NS5A RAV(s) and 4 patients had NS5B RAV(s) detected, but none had S282T RAV. The overall SVR12 rate was 86 % (86/100). At virologic failure (n = 13), 12 patients had NS5A RAV(s) but only 2 patients had new emergent NS5A RAV(s), and 6 patients had NS5B RAV(s), including the S282T RAV (n = 3), detected. Frequent AEs (>10 %) were fatigue, headache, and diarrhea, and mostly mild in severity.

Conclusions: Ledipasvir/sofosbuvir regimens were well tolerated and demonstrated that successful retreatment of SOF-failures is possible in genotype-1-infected, NS5A-naive patients and in some NS5A-experienced patients.

Table 1: Baseline Characteristics and Virologic Response by LDV/SOF Retreatment Regimen

	LDV/SOF-RBV 12 Weeks (N=51)	LDV/SOF-RBV 24 Weeks (N=8)	LDV/SOF 24 Weeks (N=41)
Baseline Characteristics:			
Age, mean (range)	54 (27-68)	61 (51-70)	58 (35-71)
Male, n (%)	31 (61)	7 (88)	34 (83)
IL28B Non-CC, n (%)	47 (92)	8 (100)	38 (93)
Log10 HCV RNA, mean (range)	6.2 (4.4-7.3)	5.6 (5.2-6.3)	6.2 (4.5-7.4)
Cirrhosis, n (%):			
• CPT-A (score 5-6)	14 (100)	3 (37)	19 (100)
• CPT-B (score 7-9)	0	5 (63)	0
HCV Genotype (GT):			
• GT 1a, n (%)	30 (59)	3 (38)	34 (83)
• GT 1b, n (%)	20 (39)	5 (63)	7 (17)
• GT 3a, n (%)	1 (2) ^a	0	0
Prior Treatment:			
SOF + Peg-IFN/RBV, n (%)	25 (49)	0	0
SOF \pm RBV, n (%)	21 (41)	8 (100)	0
LDV/SOF \pm RBV, n (%)	0	0	41 (100)
SOF placebo, n (%)	5 (10)	0	0
Resistance-Associated Variants (RAVs):			
• NS5A, n (%)	6 (12)	1 (12)	30 (73)
• NS5B, n (%)	2 (4)	1 (12)	1 (2)
Virologic Response:			
Overall SVR12, n (%)	50 (98)	7 (88)	29 (71)
Relapse, n (%)	1 (1) ^a	-	11 (28)
On-Rx Virologic Failure, n (%)	-	-	1 (2)
Visit Pending, n (%)	-	1 (12)	-

a. Genotype-3a patient enrolled in error

O-012

Asian genotype 1 HCV achieve 99 % SVR with 12 wks of LDV/SOF: analysis of phase 3 multicenter studies

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Introduction: The majority of patients with chronic HCV infection in Japan, Korea, and Taiwan are infected with HCV genotype (GT)1. The aim of this integrated analysis is to evaluate the efficacy and safety of LDV/SOF in a large cohort of Asian patients with chronic GT1 HCV-infection.

Methods: This analysis combines data from two Phase-3 trials: GS-US-337-0113 (Japan) and GS-US-337-0131 (Korea and Taiwan) evaluating 12-weeks of LDV/SOF (90 mg/400 mg) in treatment-naïve and treatment-experienced adults with chronic GT1 HCV-infection. The primary efficacy endpoint was SVR12.

Results: Overall, 349 subjects were enrolled and 67 (19 %) had cirrhosis. The majority were female (58 %), treatment-experienced (51 %), GT1b infected (94 %), and IL28B CC (62 %). The mean age (range) was 57 (18–80) years old, BMI 24 (17–38) kg/m², and HCV RNA was 6.6 (3.7–7.6) log₁₀ IU/mL. HCV NS5A RAVs were

detected in 23 % (80/343) of subjects at baseline. The overall SVR12 rate was 99 % (346/349); 2 subjects relapsed and 1 subject prematurely discontinued treatment. All treatment-experienced subjects with cirrhosis (45/45) achieved SVR12. NS5A RAVs were detected at the time of relapse but no NS5B RAVs were detected. Serious AE and treatment discontinuations were rare (<2 %). Adverse events were generally mild in severity. No significant laboratory abnormalities were observed. SVR24 will be presented.

Conclusions: A single tablet regimen of ledipasvir/sofosbuvir administered once daily for 12 weeks is highly effective and well tolerated in Asian patients with chronic GT1 HCV infection, including those with compensated cirrhosis. Prior HCV treatment experience and the presence of cirrhosis did not impact treatment response.

O-013

Ledipasvir/sofosbuvir for 12 weeks is safe and effective for patients with HBV and HCV coinfection

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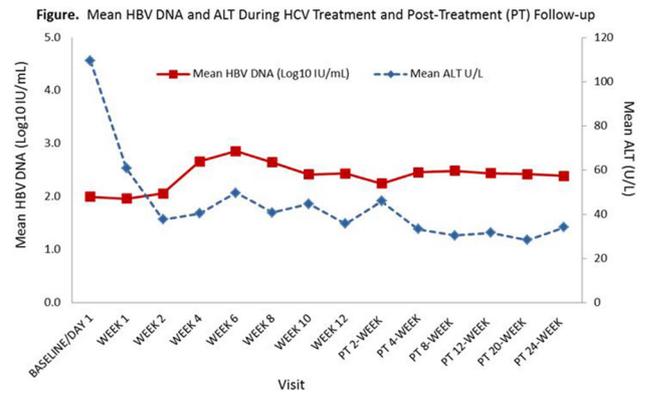
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Background: Approximately 17 million individuals worldwide are HBV/HCV coinfecting. In most patients, HCV predominates, and is associated with low to undetectable HBV DNA levels. Little is known regarding the potential reactivation of HBV when HCV is eradicated with IFN-free, direct-acting antiviral combinations. In this pilot study, we evaluated the safety and efficacy of LDV/SOF (90 mg/400 mg) once daily for 12 weeks in HBV/HCV coinfecting patients.

Methods: Genotype-1 HCV-infected patients with HBV coinfection not currently taking HBV therapy were enrolled. Serum HCV RNA, HBV DNA, HBsAg, HBeAg, chemistry, and hematology were evaluated from Baseline thru post-treatment week 24. The primary efficacy endpoint was SVR12. Safety endpoints included laboratory abnormalities and adverse events.

Results: 8 patients were enrolled; mean age of 53 years, Asian/Pacific Islanders (n = 5), male (n = 6), and non-cirrhotic (n = 6). Six were HCV genotype-1a-infected and 4 were genotype-1b-infected. The mean baseline HCV RNA was 6.5 log₁₀ IU/mL and HBV DNA was 2.0 log₁₀ IU/mL. All patients were HBeAg negative. All patients (100 %) achieved SVR12. Figure below shows the mean HBV DNA and ALT from Baseline through Post-treatment Week 24. No significant elevation in HBV DNA (>20,000 IU/mL) or ALT were observed. All AE were mild in severity.

Conclusions: LDV/SOF for 12 weeks was efficacious, safe and well tolerated in HCV-infected patients with HBV coinfection. No clinical evidence of HBV reactivation was observed in this small cohort. A larger study is ongoing to evaluate long-term safety and efficacy of LDV/SOF in a group of HBV/HCV coinfecting patients.



O-014

Safety and tolerability of elbasvir/grazoprevir in chronic hepatitis C infection

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Background: In phase 2–3 studies, elbasvir (EBR) 50 mg/grazoprevir (GZR) 100 mg ± ribavirin (RBV) achieved high rates of sustained virologic response in HCV-infected patients, including compensated cirrhotics. The purpose of this analysis was to define the overall safety profile of EBR/GZR for 8, 12, 16, or 18 weeks.

Methods: Adverse events (AEs) and laboratory abnormalities reported during therapy or ≤14 days of end of treatment were compared (EBR/GZR without RBV, n = 1033; EBR/GZR with RBV, n = 657; placebo, n = 105).

Results: A diverse population was enrolled: male, 61 %; Black/African American, 13 %; mean age, 52.6 years (11 % ≥65 years); compensated cirrhosis, 27 %; and HIV/HCV coinfection, 18 %. Addition of RBV was associated with more AEs (Table). Among patients who received EBR/GZR, there were 3 deaths (coronary artery disease, strangulated hernia, motor vehicle accident); all were unrelated to study medication. AE frequency was not associated with sex, age, cirrhosis, or HIV/HCV coinfection. Late ALT elevations (>5 × ULN in patients with normal ALT between treatment week [TW] 2–4) were noted in 0.8 % of patients, generally at/after TW8. These were typically asymptomatic, resolving with continued therapy,

scheduled end of therapy, or (in 3/1690, 0.18 %) a protocol-mandated stop of therapy, and were not associated with hyperbilirubinemia.

Conclusions: EBR/GZR ± RBV was generally well tolerated in a large, diverse patient population with low SAE rate. Fewer discontinuations due to AEs and overall improved tolerability with lower reductions in hemoglobin and lower elevations in total bilirubin were demonstrated in the RBV-free cohort. Late ALT elevations were infrequent and not clinically significant.

Parameter	EBR/GZR (no RBV)	EBR/GZR + RBV	Placebo
	n=1033	n=657	n=105
≥1 adverse event (AE)	71.4%	83.6%	68.6%
≥1 treatment-related AE	40.1%	67.6%	39.0%
Specific treatment-related AEs in >5% of patients	Fatigue (12.0%) Headache (11.5%)	Fatigue (24.7%) Headache (16.3%) Nausea (12.6%) Asthenia (9.3%) Anemia (9.1%) Insomnia (8.8%) Pruritus (8.8%) Rash (6.8%) Dyspnea (6.4%)	Fatigue (9.5%) Headache (8.6%) Pruritus (6.7%)
Serious AEs (SAEs)	2.4%	2.6%	2.9%
Treatment-related SAEs	0.1%	0.5%	0.0%
Discontinuation due to AE	0.5%	1.7%	1.0%
Discontinuation due to treatment-related AE	0.3%	0.8%	1.0%
Grade 3 ⁺ ↓ hemoglobin (Hgb)	0.0%	2.7%	0.0%
Mean Hgb decline (mg/dL) at treatment week 8	-0.3	-2.2	-0.1
Grade 3 ⁺ ↑ alanine aminotransferase	1.6%	0.6%	8.6%
Grade 3 ⁺ ↑ total bilirubin	0.3%	5.9%	0.0%

O-015

Elbasvir and grazoprevir ± ribavirin for 8 or 12 weeks in HCV GT1b-infected patients

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Background: Genotype 1b is the most common HCV genotype. We compared the efficacy of 8 vs. 12 weeks of elbasvir 50 mg/d (EBR) and grazoprevir 100 mg/d (GZR) ± ribavirin (RBV) among HCV GT1b-infected patients enrolled in the phase 2/3 EBR/GZR program.

Methods: This pooled analysis included 203 treatment-naïve (TN), noncirrhotic, HCV GT1b-monoinfected patients enrolled in the C-WORTHY and C-EDGE treatment-naïve trials (EBR/GZR ± RBV for 8 weeks, n = 61; EBR/GZR ± RBV for 12 weeks, n = 142). Absence of cirrhosis was confirmed by liver biopsy or noninvasive tests. The primary endpoint was the proportion of patients with HCV RNA below the lower limit of quantitation (LLOQ) 12 weeks after treatment (SVR12).

Results: Mean age was 52 years (range 20–78), 60 % were male, 19 % were black/African American, and 77 % had IL28B non-CC. By end of treatment at 8 or 12 weeks, 100 % of patients had HCV

RNA < LLOQ. Relapse occurred in 2 patients in each 8-week treatment group and 1 patient in each 12-week treatment group. None of the 4 virologic failures treated for 8 weeks failed with a treatment-emergent (TE) NS3 resistance-associated variant (RAV); 1 had a TE NS5A RAV. Of the 2 failures who completed 12 weeks of treatment, 1 had a TE NS3 RAV and both had a TE NS5A RAV.

Conclusions: SVR12 was >90 % in TN noncirrhotic GT1b-infected patients, regardless of treatment with RBV. Despite higher rate of relapse, treatment-emergent RAVs were detected in only 1 of 4 virologic failures treated for 8 weeks.

	HCV GT1b Infection			
	EBR/GZR + RBV (8 weeks) n=30	EBR/GZR (8 weeks) n=31	EBR/GZR + RBV (12 weeks) n=32	EBR/GZR (12 weeks) n=110
SVR12, n/N (%)	27/30 (90.0%)	29/31 (93.5%)	29/32 (90.6%)	108/110 (98.2%)
Virologic failure	2/30 (6.7%)	2/31 (6.5%)	1/32 (3.1%)	1/110 (0.9%)
Other	1/30 (3.4%)*	0	2/32 (6.3%)* [†]	1/110 (0.9%)*

*Lost to follow-up.

[†]Patient failed with GT2 reinfection.

O-016

Sofosbuvir/velpatasvir for 12 wks vs sofosbuvir + ribavirin for 24 wks in GT3 HCV: the ASTRAL-3 study

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Introduction: Velpatasvir (VEL) is a pangenotypic HCV NS5A inhibitor. In Phase 2 studies, the combination of sofosbuvir (SOF) and VEL for 12 weeks demonstrated high efficacy in patients with genotype 3 HCV. This international, multi-center, Phase 3 study compared treatment with a fixed dose combination (FDC) of SOF/VEL for 12 weeks to SOF + RBV for 24 weeks, in patients with genotype 3 HCV.

Methods: Patients at 75 sites were randomized 1:1 to receive SOF/VEL (400 mg/100 mg daily) FDC for 12 weeks or SOF (400 mg daily) with RBV (1000–1200 mg daily) for 24 weeks. The primary endpoint was sustained virologic response 12 weeks after treatment (SVR12).

Results: Of the 552 patients treated, 62 % were male, 89 % were white, 26 % had prior treatment failure, and 30 % had cirrhosis. Nine

patients, all from the SOF + RBV treatment group, discontinued treatment due to adverse events. Hemoglobin decline and total bilirubin increases were more commonly observed in the group treated with SOF + RBV consistent with RBV-induced hemolysis. No other significant lab abnormalities were observed. The SVR12 rate receiving SOF/VEL for 12 weeks was 95 % (264/277) and was statistically superior to the 80 % (221/275) SVR12 rate in patients treated with SOF + RBV for 24 weeks ($p < 0.001$).

Conclusions: The once daily, all-oral, single tablet regimen of SOF/VEL was well tolerated in treatment-naïve and treatment-experienced genotype 3 HCV-infected patients with and without cirrhosis. There were no discontinuations due to adverse events and a lower incidence of fatigue, insomnia and irritability in patients treated with SOF/VEL for 12 weeks compared to patients treated with SOF + RBV for 24 weeks.

O-017

Sofosbuvir/velpatasvir for 12 wks versus 12 wks of sofosbuvir + ribavirin in GT2 HCV: the ASTRAL-2 study

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Introduction: Velpatasvir (VEL) is a pangenotypic HCV-NS5A inhibitor. The Phase-3 ASTRAL-2 study compared the efficacy and safety of treatment with a fixed dose combination (FDC) of SOF/VEL for 12 weeks to SOF + RBV for 12 weeks in treatment-naïve and treatment-experienced genotype-2 HCV-infected patients, with and without cirrhosis.

Methods: Patients were randomized 1:1 to receive either SOF/VEL (400 mg/100 mg daily) FDC for 12-weeks or SOF (400 mg daily) with RBV (1000–1200 mg daily) for 12-weeks. The primary endpoint was sustained-virologic-response 12 weeks after treatment (SVR12).

Results: 266 patients with genotype 2 HCV infection were treated, 134 with SOF/VEL and 132 with SOF + RBV. Overall 59 % were male, 88 % were white, 15 % had prior treatment failure, and 14 % had compensated cirrhosis. The SVR12 rate for the SOF/VEL treated patients is 99 % (133/134) and for the SOF + RBV treated patients is 94 % (124/132), meeting the primary-endpoint and demonstrating statistical superiority over SOF + RBV ($p = 0.018$). There were no patients with relapse in the SOF/VEL group compared with 6 patients with relapse in the SOF + RBV treated group through post-treatment week 12. Two patients discontinued treatment (one in each arm) and AEs were more frequently observed in the SOF + RBV treated patients. Hemoglobin declined to <10 g/dL in 6(5 %) patients taking

SOF + RBV but not in patients taking SOF/VEL. No other significant laboratory abnormalities were observed.

Conclusions: Treatment with SOF/VEL FDC for 12 weeks resulted in high SVR12 rates and was well tolerated in treatment-naïve and treatment-experienced genotype 2 HCV-infected patients with and without cirrhosis. Few (<1 %) patients discontinued treatment. AEs were more common in patients treated with SOF + RBV.

O-018

Sofosbuvir/velpatasvir (SOF/VEL) for 12 weeks in genotype 1, 2, 4, 5, 6 HCV patients: ASTRAL-1 study

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Introduction: Velpatasvir (VEL) is a pangenotypic HCV-NS5A inhibitor. This Phase 3 study evaluated treatment with a fixed dose combination of SOF/VEL for 12 weeks in patients with genotype 1, 2, 4, 5, or 6 HCV infection.

Methods: Patients with genotype 1, 2, 4, or 6 chronic HCV infection were randomized 5:1 to received SOF/VEL (400 mg /100 mg daily) or placebo for 12 weeks. Patients with genotype 5 infection were enrolled to the SOF/VEL treatment group and patients with genotype 3 were evaluated in a separate study.

Results: 740 patients were enrolled at 81 international sites: 60 % male, 79 % white, 32 % treatment-experienced (TE), and 19 % compensated-cirrhosis. Of the 624 patients treated with SOF/VEL, the genotype distribution was 53 % GT1, 17 % GT2, 19 % GT4, 6 % GT5 and 7 % GT6. Overall SVR12 for SOF/VEL-treated patients was 99.0 % and the study met its primary efficacy endpoint. SVR12 rates by HCV genotype are presented in the table. Two of 328 patients (0.6 %) with genotype-1 infection had virologic relapse. No patients with genotype 2, 4, 5, or 6, including 48 with cirrhosis, had virologic failure. Four patients did not achieve SVR12 for non-virologic reasons. AEs and laboratory abnormalities were similar in the SOF/VEL-

treated patients compared with the 116 placebo-treated patients. One patient discontinued SOF/VEL treatment due to adverse-events.

Conclusions: Treatment with the once daily, all-oral, single tablet regimen of SOF/VEL for 12 weeks is well tolerated and results in high SVR12 rates in treatment-naïve and treatment-experienced genotype 1,2,4,5,6 HCV-infected patients with and without cirrhosis.

ASTRAL-1 : SVR12 rates by HCV genotype

HCV Genotype	Total (N = 624)	GT-1 (N = 328)	GT-2 (N = 104)	GT-4 (N = 116)	GT-5 (N = 35)	GT-6 (N = 41)
Cirrhosis %, (n/N)	19.4% (121/624)	22.3% (73/328)	9.6% (10/104)	23.3% (27/116)	14.3% (5/35)	14.6% (6/41)
SVR12 %, (n/N)	99.0% (618/624)	98.5% (323/328)	100.0% (104/104)	100.0% (116/116)	97.1% (34/35)	100.0% (41/41)

O-019

Serum levels of Mac-2 binding protein and the associated risk for HCC among CHC patients

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Background and aim: The current study aimed to evaluate *Wisteria floribunda* agglutinin-positive human Mac-2 binding protein (WFA + -M2BP) and its association with other factors and risk for hepatocellular carcinoma (HCC) among chronic hepatitis C (CHC) patients.

Methods: The study subjects were a subgroup of R.E.V.E.A.L.-HCV study, which enrolled participants in 1991 and annually followed-up until 2005. We retrieved two serial samples of the subjects: the samples at study entry and at the last follow-up. The serial samples nearest to HCC diagnosis of HCC cases and the samples at the last follow-up of controls were retrieved. The time interval between the baseline and the last follow-up was matched for cases and controls. There were 62 HCC cases and 248 controls for subsequent analyses.

Results: Elevated baseline serum level of WFA⁺-M2BP occurred more often in the elderly, females, persons without smoking or alcohol consumption, persons having higher BMI, higher serum level of AST, ALT and HCV RNA ($p < 0.05$). Persons with elevated WFA + -M2BP at study entry were more likely to experience HCC development. The adjusted OR (95 % CI) was 2.00 (0.91–4.40) for 0.8–1.66 COI and 3.38 (1.23–9.33) for >1.66 COI, by using WFA + -M2BP <0.8 COI as a reference group (p for trend <0.05). The elevated WFA + -M2BP at the last follow-up were found among HCC cases compared to controls. However, it was not significantly associated with HCC risk after adjustment of HCV RNA ($p > 0.05$).

Conclusions: M2BP may be applied as a long-term useful predictor for HCC among chronic hepatitis C patients.

O-020

Reappearance of hepatitis C virus accompanied by genotypic change after sustained viral response

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We report two cases of late relapse of hepatitis C virus (HCV) after interferon treatment. Relapsed HCV had different genotypes from those before the treatment.

Case 1: A 56-year-old woman infected with HCV serotype 2 and genotype 2b received 16 weeks of peginterferon plus ribavirin treatment, and sustained viral response (SVR) was achieved 24 weeks after completion of treatment. One year and 3 months after SVR, HCV RNA reappeared at 4.0 log IU/ml, and the serotype and genotype were 1 and 1a, respectively.

Case 2: A 50-year-old man infected with HCV GT 1b received 48 weeks of peginterferon plus ribavirin treatment, and SVR was achieved 24 weeks after completion of treatment. Six months after SVR, HCV RNA reappeared at 6.3 log IU/ml and the genotype was 2a. Medical interviews ruled out HCV infection through common routes such as intravenous drug use, tattoos, and unsafe sexual practices for both patients.

Discussion: Late relapse is defined as reappearance of HCV after achieving SVR. When the serotype or genotype of the relapsed HCV is different from that before SVR, it is usually called a reinfection. HCV reinfection is often seen in individuals exhibiting risk-taking behaviors, which was not true for our cases. In addition, in recent years, acute HCV infection without risk-taking behaviors has been rare in Japan, except those due to needle injury. Our cases imply that different HCV genotypes may coexist in some patients and that latent infection with minor genotype can cause late relapse.

O-021

Cost-effectiveness of DAAs for CHC in affluent countries of Asia-Pacific region

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Background and aim: The Asia-Pacific region bears the highest burden of world's hepatitis infections, but its access to direct-acting antiviral (DAA) drugs is low. The most significant barriers are price of drugs and purchasing power in countries of AP region. We aim to evaluate cost-effectiveness of DAAs in five affluent places in AP region.

Methods: We conducted the analysis in Australia, Japan, Singapore, Hong Kong and Taiwan. Decision analytic Markov models were developed for each place to estimate health outcome in quality-adjusted-life-years, lifetime cost of HCV infection and incremental cost-effectiveness-ratios (ICERs) by genotype, treatment history and fibrosis stage. Real-life data was used whenever it was available from each place.

Results: According to GDP per capita and treatment strategies in each place, DAAs were cost-saving or very cost-effective (ICERs < one GDP per capita) in Japan and Australia across different genotypes. DAAs were very cost-effective and cost-effective (ICERs < three GDP per capita) for HCV genotype 1a, 1b and 6 in Singapore with ICERs from US\$14,091 to US\$109,116 and in treatment-experienced patients in Taiwan (ICERs = US\$19,140 to US\$52,324). DAAs were cost-effective for genotype 1b, 3 and 6 in treatment-experienced patients in Hong Kong. The probability of cost-effectiveness varied widely in each place (Japan, 39–92 %; Australia, 24–74 %; Singapore, 14–82 %; Taiwan, 38–56 %; Hong Kong, 16–80 %).

Conclusion: DAAs present different values for money in affluent places in AP region depending on their purchasing power and acquisition price of drugs. Differential prices and government subvention are needed to maximize accessibility and increase affordability to DAAs.

O-022

12 W of sofosbuvir/simeprevir in genotypes 1 and 4 in HCV, cirrhotic patients: Qatar experience

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Data for treating GT 1 and 4 patients with sofosbuvir plus simeprevir are limited. Our aims are to assess the tolerability and effectiveness of 12 weeks regimen of SOF/SMV without Ribavirin to treat naive and experienced, cirrhotic, genotypes 1 and 4 patients, and the effect of BMI? 30 and IL 28B, on response.

Methods: 31 patients with advanced fibrosis have received 12 weeks of SOF/SMV therapy. 14/31(45.2 %) and 17/31(54.8 %) patients were genotype 1 and 4 respectively.

Results: 71 % of patients had cirrhosis (n = 22) and 80.6 % were previously treated with PR (n = 25). From the total population, 41.9 % (n = 13) were Child Pugh B/C. Single patient underwent liver transplantation. 30 patients completed 12 weeks, while one discontinued at week 4 due to viral breakthrough. The 12 weeks SOF/SMV achieved SVR12 rate of 90 % in patients with cirrhosis. 86 and 100 % were SVR12 in G1 and G4 respectively. Signs of decompensation

occurred in 2 Child Pugh C patients, however, achieving SVR. Side effects were skin rash, elevated bilirubin level and elevated albumin level in the first 4 weeks.

Conclusions: short course of oral sofosbuvir plus simeprevir for 12 weeks without ribavirin achieved high SVR and well tolerated in both Child A and B patients, including experienced patients with prior null response, in both genotypes 1 and 4. Cautious should be considered when using this regimen in Child Pugh C patients. Unlike convention therapy, neither BMI nor Non C/C IL28b polymorphism had an impact on response to SOF/SMV therapy.

O-023

36 weeks of sofosbuvir-ribavirin combination therapy for Egyptians with HCV genotype 4

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Background: The Egyptian government has initiated a strategic national program for treating all patients with HCV, aiming at total eradication of HCV within 10–15 years. For non-interferon eligible patients, Sofosbuvir-Ribavirin 24 weeks of therapy was the alternative option. Considering that longer anti-viral suppression therapy, yields better results. We have decided to assess the efficacy of longer course of treatment (36 weeks).

Trial design: This was a prospective, open-label, multicenter, two-arm study.

Patients and method: All patients, (50) were Egyptians infected with HCV genotype 4, with fibrosis grade F3 or F4. Both groups were of comparable age, gender, fibrosis scores and BMI.

Methods: 50 patients were studied, 25 of them received Sofosbuvir (GILEAD), 400 mg once/day and Ribavirin for 24 weeks (Group 1) and 25 patients were treated for 36 weeks. (Group 2) HCV PCR was tested at weeks, 4, 12, 24, 36, 48.

Results: All patients (except 4) had undetectable HCV RNA after 4, 12, and 24 weeks of therapy indicating EOT response rate of 92 % (46/50). Four cases, (2 in each group), have their treatment stopped due to the detection of HCV PCR at weeks 4 and/or 12 respectively. Three patients in Group 1 have their HCV PCR detected at week 36, referring to a relapse rate of 12 % (3/25) while HCV RNA remained undetectable in the other 23 patients of Group 2 at weeks 36 and 48.

Conclusion: Treating HCV patients, genotype 4 by Sofosbuvir-Ribavirin combination course for 36 weeks, is more effective than 24 weeks of therapy.

O-024

Sofosbuvir and ledipasvir versus sofosbuvir and simeprevir for acute hepatitis C: a RCT:SLAM C study

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Objectives: Acute hepatitis C has been increasing in incidence recently in the USA. Traditional management with peg IFN alpha 2a and weight based RBV has had equivocal success with SVR rates as low as 30 % in some studies and considerable side events. Recently oral DAAs have achieved SVR rates exceeding 95 % in many cohorts.

Aim: This clinical pilot study evaluates the efficacy of Sofosbuvir (SOF) with Ledipasvir (LDV) or Simeprevir (SIM) in acute Hepatitis C. **Methods:** 29 patients with a diagnosis of acute hepatitis C (negative past HCV antibody + new onset HCV RNA) were recruited from 6 inner city drug rehab programs in Brooklyn, NY. They were divided into 2 groups; Group A (n = 14): SOF 400 mg + LDV 90 mg, daily once, for 4 weeks Group B (n = 15): SOF 400 mg + SIM 150 mg, daily once, for 8 weeks.

Results: This study demonstrates a high SVR with short-course DAAs in acute hepatitis C with SVR (at 20 weeks) greater than 90 % in both groups. **Conclusion:** This study demonstrates a high SVR with short-course DAAs in acute hepatitis C with SVR (at 20 weeks) greater than 90 % in both groups. The drugs were well tolerated.

O-025

Outcomes of sofosbuvir and ribavirin for the treatment of HCV recurrence after liver transplantation

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Background: Recurrent hepatitis occurs in the vast majority of HCV-positive recipients who are viremic at the time of Liver Transplantation (LT). We evaluated safety and efficacy of all oral antiviral therapy (Tx) with Sofosbuvir plus Ribavirin in a population of HCV G3-infected patients.

Methods: We prospectively enrolled 30 liver transplant patients who had recurrent hepatitis. They were HCV RNA positive with fibrosis score F3 (21pts) or F4 (9pts) according to Fibroscan, 70 % male, mean-age 50 years, mean BMI 25. All patients were G3, 43 % were receiving mycophenolate, 47 % tacrolimus. Patients received SOF 400 mg/daily plus RBV (weight based: 1000 mg daily if <75 kg; 1200 mg daily if ≥75 kg) for 24 weeks. Median time from LT to Tx 4.96 years (0.25–14.25); mean baseline HCV RNA 6.62 log₁₀ IU/mL (3.4–7.9 log₁₀ IU/mL); mean GFR 76 mL/min.

Results: All 30 patients completed 24-weeks of treatment. All 30 patients were HCVPCR negative at week-4, week-12 and 29/30(96 %) at week 24. The most common adverse events were fatigue, headache and nausea. During Tx, 1 patient had variceal bleeding (day 100) and 1 died due to multi-organ failure (day 120); 25 % received blood transfusion or epoetin; 1 patient temporally discontinued RBV because of skin rash. Minimal immunosuppression dose adjustments were required on Tx and no rejection episodes were recorded.

Conclusions: In pts with severe HCV recurrence post-LT, an oral antiviral regimen using SOF plus RBV is very well tolerated and easy to manage, while allowing rapid HCV clearance. This is our ongoing study. Patients are under regular review to observe SVR.

O-026

Sofosbuvir plus ribavirin in the treatment of chronic HCV infection in India

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Background and aims: The burden of disease due to HCV in India is substantial, with an estimated 12 to 24 million people infected. This ongoing study is evaluating the safety and efficacy of sofosbuvir (SOF) plus ribavirin (RBV) in patients in India with chronic genotype (GT) 1 or GT3 HCV infection, the most common genotypes in India. **Methods:** In this open-label study, treatment-naïve patients were randomized to receive SOF (400 mg daily) + RBV (1000–1200 mg daily) for 16 or 24 weeks; randomization was stratified by genotype and by presence or absence of cirrhosis. The primary endpoint is sustained virologic response 12 weeks post-treatment (SVR12).

Results: 117 patients (58 GT1 and 59 GT3) were enrolled at 14 sites: 64 % were male, 41 % had non-CC IL28B genotype, 28 % had compensated cirrhosis, and 70 % had baseline HCV RNA ≥800,000 IU/mL. SVR12 rates were 90 % (27/30) in GT1 and 100 % (29/29) in GT3 HCV with 16 weeks SOF + RBV and 96 % (27/28) and 93 % (28/30), respectively, with 24 weeks SOF + RBV. All treatment failures were due to relapse except 1 GT3 patient lost to follow-up. The most common AEs (≥10 % of patients) were asthenia, headache, cough, fatigue, and dyspepsia. Most AEs were mild or moderate. Two patients had serious AEs that were unrelated to treatment; 1 SAE (urinary retention) resulted in treatment discontinuation.

Conclusions: An interferon-free regimen of SOF + RBV for 16 or 24 weeks resulted in SVR12 rates ≥90 % in treatment-naïve patients in India with GT1 or GT3 HCV. SOF + RBV was well tolerated.

	SVR12 % (n/N)			
	GT1		GT3	
	SOF+RBV 16w	SOF+RBV 24w	SOF+RBV 16w	SOF+RBV 24w
Overall	90 (27/30)	96 (27/28)	100 (29/29)	93 (28/30)
Cirrhosis				
Present	80 (8/10)	89 (8/9)	100 (7/7)	86 (6/7)
Absent	95 (19/20)	100 (19/19)	100 (22/22)	96 (22/23)
IL28B genotype				
CC	86 (12/14)	100 (15/15)	100 (17/17)	91 (21/23)
Non-CC	94 (15/16)	92 (12/13)	100 (12/12)	100 (7/7)

O-027

Sofosbuvir regimens are safe and highly effective in patients with hereditary bleeding disorders

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Background: Patients with bleeding disorders constitute a special population, have a high prevalence of Hepatitis C Virus (HCV) infection and historically have been excluded from clinical trials. We evaluated the safety and efficacy of ledipasvir/sofosbuvir (LDV/SOF, genotype [GT] 1 and 4) and SOF + RBV (GT2 and 3) in patients with bleeding disorders.

Methods: GT1 or 4 HCV-infected patients received LDV/SOF (90 mg/400 mg) fixed dose combination daily for 12 weeks; treatment was extended to 24 weeks for those who were treatment experienced with cirrhosis. GT2 or GT3 HCV-infected patients received SOF400 mg daily + RBV (1000 or 1200 mg/day divided BID) for 12 weeks or 24 weeks, respectively.

Results: Thirteen sites in the US enrolled 120 patients (103 GT1, 10 GT2, 6 GT3, 1 GT4). Bleeding disorders included Hemophilia-A (65 %) and Hemophilia-B (26 %), Type-1 or 3 Von-Willebrand's Disease (3 % each), Type-2 Von Willebrand's Disease (2 %), and Factor XI deficiency (<1 %). One patient was lost to follow up and there were no virologic failures. Adverse events were generally mild, and Grade 3/4 laboratory abnormalities were infrequent. Mostly mild or moderate severity hemorrhagic events occurred in 22 patients (18 %). See Table for SVR data and more complete data will be presented

Conclusions: The interferon-free regimens of LDV/SOF and SOF + RBV led to high rates of SVR in genotype 1-4 HCV-infected patients with bleeding disorders with and without HIV-coinfection. Both regimens were safe and well-tolerated; no new toxicities specific to patients with bleeding disorders emerged. All-oral SOF-based regimens offer an important therapeutic option for this group of patients with a high unmet need.

Table 1. Demographics, Baseline Characteristics and Efficacy by Genotype of LDV/SOF and SOF+RBV in patients with hereditary bleeding disorders

	Genotype 1 or 4 (LDV/SOF) 12 weeks	Genotype 1 or 4 (LDV/SOF) 24 weeks	Genotype 2 (SOF + RBV) 12 weeks	Genotype 3 (SOF + RBV) 24 weeks
Number	99	5	10	6
Cirrhosis, n (%)	27 (27)	5 (100)	2 (20)	2 (33)
HIV Positive, n (%)	19 (19)	0	4 (40)	3 (50)
Treatment Experienced, n (%)	39 (39)	5 (100)	3 (30)	1 (17)
IL28B CC, n (%)	21 (21)	2 (40)	7 (70)	5 (83)
SVR12, n (%)	98 (99)	5 (100)	10 (100)	5 (83)

O-028

Higher quality of life gains in Japanese hepatitis C patients treated with ledipasvir/sofosbuvir

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Background: Cross-cultural differences in health-related quality of life (HRQOL) may be important for clinical trials chronic hepatitis C (CHC) patients enrolled from different regions of the world. Our aim was to compare HRQOL of Japanese CHC patients to CHC patients from North America treated with sofosbuvir (SOF)-based regimens with and without ribavirin (RBV).

Methods: The HRQOL data (Short Form-36) was collected in two multicenter phase 3 clinical trials of RBV-free and RBV-containing regimens conducted in Japan. We used historical controls of North American patients treated with the same regimens (matched by age, gender, cirrhosis, diabetes, treatment history, and history of psychiatric disorders).

Results: There were 338 Japanese CHC patients (50 % male, 18 % cirrhotic, and 57 % treatment-naïve) with similar matched American CHC patients (N = 338). Despite extensive matching, baseline HRQL scores in Japanese patients were higher in a number of physical health-related domains while their general health scores were lower (all p < 0.006). During treatment with RBV-containing regimens, Japanese and American patients experienced similar moderate decrements in HRQOL scores (all p > 0.05) except for the vitality score (no decrement in Japanese patients). On the other hand, during treatment with RBV-free regimens (LDV/SOF), Japanese patients with CHC experienced greater improvements in vitality and bodily pain scores compared to American patients (p < 0.05). Post-treatment and after sustained virologic response-12 (SVR-12), both patient groups experienced similar improvement of their HRQOL.

Conclusions: Despite higher HRQOL scores at baseline, Japanese CHC patients experience higher HRQOL gains during treatment with LDV/SOF. Furthermore, they experience improvement of HRQOL scores after achieving SVR.

O-029

High therapeutic efficiency of LDV/SOF in Asian patients with CHC genotype 1 infection

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Background: Current Asian treatment practices for Chronic Hepatitis C (CHC) Genotype (GT) 1 patients use regimens containing pegylated interferon and ribavirin (PR). As interferon-free regimens become the standard of care in most Western countries, it is necessary to understand the potential impact of an all-oral, PR-free single-tablet regimen of Ledipasvir/Sofosbuvir (LDV/SOF) on Asian CHC patients. The aim of this study was to estimate long-term health outcomes of LDV/SOF therapy in 4 Asian countries: Taiwan, South Korea, Singapore, Hong Kong. **Methods:** A hypothetical cohort of 10,000 adult patients/country was modeled with a hybrid decision tree and Markov state-transition model capturing the natural history of CHC and treatment implications over a lifetime. Efficacy was based on randomized controlled trials; country-specific demographics, HCV-related epidemiology and treatment data were retrieved from literature. Therapeutic efficiency was defined as the number of advanced liver disease (ALD) cases averted (decompensated cirrhosis, hepatocellular carcinoma, liver transplants, HCV-related deaths) with LDV/SOF relative to PR or no treatment (NT) in treatment-naïve patients. The differing immunomodulatory and anti-tumor effects of the therapies were not modeled. **Results:** A 12 week regimen of LDV/SOF compared to PR/NT is estimated to substantially impact CHC disease burden by reducing the incidence of ALD (Table 1): -90.6 %/-94.2 % vs. PR/NT (Taiwan), -92.5 %/-95.7 % (South Korea), -93.3 %/-96.2 % (Singapore), -93.4 %/-96.3 % (Hong Kong). **Conclusions:** LDV/SOF is a highly effective treatment associated with potentially more favorable health outcomes when compared with current treatment practices or no treatment for GT1 CHC Asian patients.

Table 1. Long-Term Health Outcomes of No Treatment vs PegIFN+RBV vs LDV/SOF

	Regimen	Number of new cases in a n=10,000 cohort				
		DCC	HCC	LT	HCV-related deaths	Overall advanced liver Disease
Taiwan	LDV/SOF 12 wks	73	168	10	203	454
	PR	1,702	1,261	222	1,622	4,808
	No treatment	2,697	1,954	343	2,799	7,792
Korea	LDV/SOF 12 wks	72	163	11	203	449
	PR	2,014	1,491	281	2,197	5,984
	No treatment	3,404	2,479	469	4,113	10,466
Singapore	LDV/SOF 12 wks	69	161	11	203	443
	PR	2,168	1,607	316	2,517	6,608
	No treatment	3,681	2,687	529	4,681	11,578
Hong Kong	LDV/SOF 12 wks	70	163	11	206	449
	PR	2,228	1,651	328	2,621	6,828
	No treatment	3,794	2,770	551	4,887	12,001

O-030

Cost-utility analysis of ledipasvir/sofosbuvir for treatment of GT 1 chronic hepatitis C in Japan

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Background: An estimated 1.5–2 million people are infected with HCV in Japan, approximately 70–80 % of which are genotype 1b. Japanese patients are older with frequent co-morbidities thereby

limiting use of pegylated interferon (PEGIFN) and ribavirin (RBV)-based regimens. The once-daily and IFN-free single tablet regimen of Sofosbuvir (SOF) and ledipasvir (LDV) has shown a 100 % sustained virological response (SVR) in Japanese Phase III trials. However, there are growing concerns regarding cost implications of LDV/SOF. Hence, our objective was to conduct cost-utility analysis of LDV/SOF in GT1 patients in Japanese setting.

Methods: A cohort of 10,000 patients was followed through a lifetime Markov model, accounting for treatment-naïve (TN) and treatment-experienced (TE) populations, both non-cirrhotic (NC) and cirrhotic (CC). LDV/SOF was compared to several treatment regimens, including PEGIFN and RBV-containing regimens [telaprevir and simeprevir] and the IFN-free combination of daclatasvir/asunaprevir. Japanese costs/outcomes data were adopted as much as possible. Quality-Adjusted Life Years (QALYs) was set as the primary outcome measure.

Results: LDV/SOF resulted in significantly lower cases of advanced liver disease when compared to other treatment options. From healthcare payers' perspective, LDV/SOF was cost-effective in 15 of the 16 base case comparisons with incremental cost-effectiveness ratios (ICERs) below hypothetical threshold value, or the JPY5Million/QALY gained. Under a societal perspective, LDV/SOF was the dominant treatment strategy in all cases (Table 1). Results were robust to sensitivity analyses.

Conclusions: LDV/SOF is a cost-effective treatment for Japanese CHC patients; it also extends treatment to patients ineligible for IFN-based regimens. Hence, LDV/SOF can potentially help reduce HCV burden.

Table 1. Summary of results for treatment of patients with GT1 CHC

Indication	Comparator	Difference total direct costs	QALY gained	CC avoided	DCC avoided	HCC avoided	LT avoided	ICER (JPY/QALY)	ICER (JPY/QALY) with indirect costs included
GT1 TN NC	TVR + PR2b	2,611,096	1.86	2,242	925	1,301	9	1,405,156	Dominant
	SMV + PR2b	3,754,282	0.82	692	286	402	3	6,097,686	Dominant
	DCV + ASV	3,370,457	0.9	1,072	442	622	5	3,742,906	Dominant
	PR2b	765,744	3.79	4,709	1,936	2,723	20	201,876	Dominant
	No treatment	113,722	6.77	8,329	3,442	4,846	35	16,799	Dominant
GT1 TN CC	DCV + ASV	3,473,187	1.22	0	349	697	4	2,853,483	Dominant
	PR2b	-200,350	7.42	0	2,240	4,391	25	Dominant	Dominant
	No treatment	887,745	9.46	0	2,787	5,454	31	93,868	Dominant
	TVR + PR2b	3,198,836	1.67	2,005	827	1,165	8	1,916,863	Dominant
	SMV + PR2b	2,274,180	2.12	2,568	1,059	1,491	11	1,072,340	Dominant
GT1 TE NC	DCV + ASV	2,908,240	1.44	1,749	721	1,015	7	2,015,836	Dominant
	PR2b	1,414,147	3.15	3,888	1,598	2,249	16	448,913	Dominant
	No treatment	113,722	6.77	8,329	3,442	4,846	35	16,799	Dominant
	DCV + ASV	3,072,351	1.98	0	577	1,141	7	1,553,458	Dominant
	PR2b	1,080,385	5.41	0	1,621	3,190	18	199,841	Dominant
GT1 TE CC	PR2b	1,080,385	5.41	0	1,621	3,190	18	199,841	Dominant
	No treatment	887,745	9.46	0	2,787	5,454	31	93,868	Dominant

ASV: Asunaprevir; CC: Compensated cirrhosis; CHC: Chronic Hepatitis C; DCC: Decompensated cirrhosis; DCV: Daclatasvir; GT: Genotype; HCC: Hepatocellular Carcinoma; ICER: Incremental cost-effectiveness ratio; LDV: Ledipasvir; LT: Liver Transplant; LY: Life Year; PR2b: Pegylated interferon alpha2b + Ribavirin; QALY: Quality-Adjusted Life Year; SMV: Simeprevir; SOF: Sofosbuvir; TE: Treatment-experienced; TN: Treatment-naïve; TVR: Telaprevir; wks: Weeks.

O-031

Impact of ledipasvir/sofosbuvir on the work productivity of chronic hepatitis C patients in Asia

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Aim: To estimate the work productivity gains associated with LDV/SOF treatment for CHC in Hong Kong, Singapore, South Korea and Taiwan.

Methods: The model captures anticipated impact of LDV/SOF on productivity loss over a 1-year time horizon from a societal perspective for each country. A literature review was performed to identify country-specific inputs and expert advice was solicited to verify key variables. Patients enter the model post-treatment, having achieved SVR12, or not. Absenteeism and presenteeism rates were estimated based on the Work Productivity and Activity Index-Specific Health Problem (WPAI-SHP) data collected from the Phase III ION trials (US participants only) at baseline and at 12 weeks with rates assumed to remain unchanged from baseline for patients not achieving SVR. Sensitivity analyses were performed on key variables. **Results:** Total annual work productivity loss due to not treating CHC was highest in Taiwan at US\$349 M (\$355 per capita) given high prevalence of HCV, followed by US\$146 M (\$358) in Korea, US\$17 M (\$914) in Singapore and US\$11 M (\$351) in Hong Kong. Treatment with LDV/SOF resulted in estimated annual productivity gains of \$138 million, \$58.7 million, \$6.8 million and \$4.5 million in Taiwan, Korea, Singapore and Hong Kong respectively.

Conclusions: CHC imposes a significant indirect economic burden. Our model demonstrates that treatment of HCV GT1 patients with LDV/SOF is likely to result in significant cost savings due to an improvement in presenteeism versus no treatment across 4 Asian countries. This indirect economic gain should be considered when assessing the benefits of treating CHC.

O-032

Ledipasvir/sofosbuvir reduces risk factors associated with HCC in patients with chronic hepatitis C

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Background and aim: High rates of sustained virologic response have been observed among patients with chronic hepatitis C virus infection who received 12 weeks of treatment with sofosbuvir (SOF) combined with ledipasvir (LDV) ± ribavirin (RBV) for genotype (GT)-1 infection. The aim of this study was to investigate whether risk factors of hepatocellular carcinoma (HCC) were similarly reduced by LDV/SOF ± RBV compared with peginterferon (PegIFN) and RBV. **Methods:** Forty Japanese patients with GT-1 received LDV (90 mg)/SOF (400 mg) with (n = 19) and without (n = 21) RBV for 12 weeks at our hospital as part of a multicenter Phase 3 study. Serum AFP, ALT, Insulin, Ferritin, and plasma hemoglobin levels were collected and measured at baseline (BL), week (W) 12 of treatment, and post-treatment Weeks 12 and 24. Fifteen patients in our hospital who received PegIFN/RBV/Simeprevir for 24 weeks and achieved SVR served as control subjects. We also measured liver stiffness by Fibroscan (LSM) and Wisteria floribunda agglutinin-positive Mac-2

binding protein (WFA + -M2BP) for evaluation of fibrosis at BL, W12, and post-treatment Weeks 12 and 24.

Results: All patients achieved SVR24. AFP, ALT, LSM and WFA + -M2BP levels were significantly reduced at W12 and post-treatment weeks 12 and 24 compared to BL (P < 0.05) at a similar level as control subjects. Insulin and Serum Ferritin levels also decreased at post-treatment week 12 (p < 0.05). Plasma hemoglobin levels reduced at post-treatment weeks 24 for the decreased Ferritin. **Conclusions:** The risk factors of HCC improve following only 12-week LDV/SOF regimen to a similar degree as achieved with PegIFN/RBV/Simeprevir.

O-033

LDV/SOF 12 wks in a real-life population of GT 1a and 1b HCV patients; data from the TRIO network

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Background: DAA therapies such as ledipasvir/sofosbuvir (LDV/SOF) have yielded SVR12 rates over 95 % in clinical trials but need to be confirmed in real life clinical practice.

Methods: In this retrospective analysis, Data were collected from providers and specialty pharmacies through Trio Health's Innervation Platform, a cloud-based disease management program. All genotype 1 HCV patients who initiated treatment with 12 week LDV/SOF between Oct 2014 and Mar 2015 were included in the analysis (n = 1438). No patients were treated with ribavirin. Patient demographics, characteristics and SVR 12 data was collected for all genotype 1 patients.

Demographics: 1438 patients, 1010 (70 %) GT 1a patients and 428 (30 %) GT 1b patients with a mean VL of 4,567,771 IU/ml were included. Mean age 60, 57 % male and 30 % with cirrhosis. Prior treatment experience was seen in 526 patients (37 %) including PI and DAA in 96 patients (7 %).

Results: SVR was achieved overall in 1367 patients (95 %). Only 39 patients (3 %) relapsed with the remainder either lost to follow up (20 patients 1 %) and 12 patients discontinued (1 %). SVR was 95 % (853/897) in naive patients, 95 % (499/526) in treatment failures, 94 % (951/1010) in genotype 1a and 97 % (416/428) in genotype 1b. The SVR in cirrhotics was 92 % (389/425) compared to non cirrhotics 97 % (946/980). Analysis of variable predicting response showed that there were several predictors of response: Gender (p = 0.048), Practice Type (p = 0.011), and Platelet count (p = 0.00000010).

Conclusion: Real life SVR with LDV/SOF is comparable to clinical trials and seen across all patient demographic groups.

O-034

Sofosbuvir plus ribavirin treatment in genotype 2 infection in Japanese real world

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Backgrounds: Interferon free protocol has been recommended as the first line therapy for HCV infection. However, currently, Sofosbuvir (SOF) plus Ribavirin (RBV) has been the only therapeutic option for the treatment of HCV genotype 2 infection in Japan. In preceding Japanese phase 3 trial, the SVR rate was 97 %, which was significantly higher than previous interferon based standard of care. However, the usefulness of SOF/RBV has not been evaluated in real world settings in Japan. Thus, in the current study, we have evaluated the effects of SOF/RBV in Yamagata area, northern-east region in Japan.

Methods: In this study, we have registered 29 patients with HCV genotype 2 infection. The patients' backgrounds were: age (median) 68.0, gender (F/M = 17:12) cirrhosis: non-cirrhosis = 5:24, genotype 2a:2b = 12:13, and treatment history naive: relapse: nonresponder: IFN intolerant or ineligible = 4:7:8:5.

Results: All 29 patients have been successfully treated with SOF/RBV. HCV-RNA rapidly dropped after 4 weeks from the initiation of the therapy (6.3 to 0.0 log). Serum transaminase as well as serum albumin levels ameliorated after induction of SOF/RBV.

Conclusions: IFN-free regimen, SOF/RBV was highly effective in real-world setting in Japanese HCV genotype 2 infection.

O-035

Daclatasvir + sofosbuvir + ribavirin for HCV GT-3 with cirrhosis or advanced fibrosis: ALLY-3 + study

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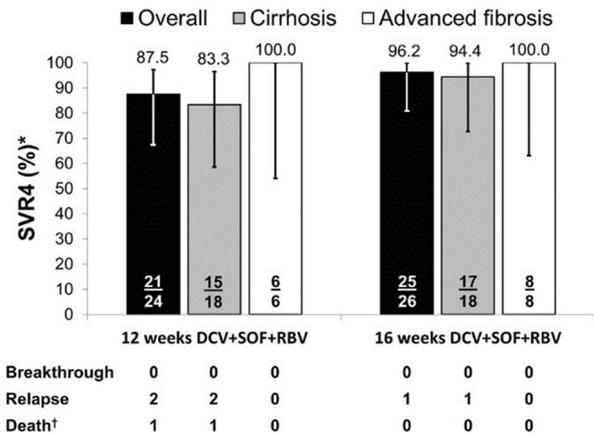
Background: HCV GT3-infected patients are a challenging population in urgent need of optimally effective therapies. A previous study (ALLY-3) in GT-3 infection achieved 96 % SVR12 in patients without cirrhosis, and 63 % in patients with cirrhosis, following 12 weeks of daclatasvir (DCV) + sofosbuvir (SOF). ALLY-3 + evaluated DCV + SOF + ribavirin (RBV) for 12 or 16 weeks in HCV GT-3 with advanced fibrosis/cirrhosis.

Methods: Open-label, phase 3b study in GT-3 treatment-naive or -experienced adults with compensated advanced fibrosis or cirrhosis. Patients were randomized 1:1 to 12 or 16 weeks of DCV (60 mg QD) + SOF (400 mg QD) + RBV (weight-based), stratified by advanced fibrosis/cirrhosis status. Interim efficacy (SVR4) and safety outcomes are reported. SVR12 (primary endpoint) data will be available for presentation.

Results: 50 patients were treated. Most were male (80 %), white (98 %), and treatment-experienced (74 %; 10 % prior relapse on SOF + RBV); 72 % had cirrhosis, and 52 % had HCV-RNA \geq 6 million IU/mL. Overall SVR4 by intention-to-treat analysis was 92 %. SVR4 in the 12-week arm was 88 % (cirrhosis 83 %, advanced fibrosis 100 %) and in the 16-week arm was 96 % (cirrhosis 94 %, advanced fibrosis 100 %; Figure); 4/5 (80 %) with prior relapse on

SOF + RBV achieved SVR4. There were 3 relapses, no virologic breakthroughs, and 1 treatment-unrelated death. Treatment was well tolerated—the most common adverse events (AEs) were insomnia (30 %), fatigue (26 %) and headache (24 %). There were no discontinuations for AEs.

Conclusion: DCV + SOF + RBV for 12 or 16 weeks achieved high SVR4 rates of 88 and 96 %, respectively in HCV GT3-infected patients with compensated advanced fibrosis/cirrhosis, and was generally well tolerated.



*HCV RNA $<$ LLOQ_{TDIND} (next-observation-carried-backward for missing data)

†Not related to treatment. Bars represent 95% confidence intervals.

O-036

GIFT-I: ALT changes with ombitasvir/paritaprevir/ritonavir and concomitant use of hepatoprotectives

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Background: To evaluate alanine aminotransferase (ALT) patterns in noncirrhotic HCV genotype 1b Japanese patients treated with coformulated OBV/PTV/r 25/150/100 mg QD (PTV identified by AbbVie and Enanta) who used concomitant hepatoprotective medications (HPM+) or not (HPM-) and who achieved sustained virologic response at post-treatment week 12 (SVR12+) or not (SVR12-).

Methods: Non-cirrhotic adult Japanese patients with HCV GT1b infection were randomized 2:1 to receive double-blind (DB) OBV/PTV/r for 12 weeks or DB PBO for 12 weeks followed by open-label (OL) OBV/PTV/r for 12 weeks. ALT was measured during OBV/PTV/r treatment. Treatment-emergent adverse events (TEAE) from first dose through 30 days after last dose were summarized for any patient who received at least one dose of study drugs.

Results: 57.9 % (186/321) of the non-cirrhotic patients used HPM. SVR12 rates were similarly high in both HPM+ (93.5 %) and HPM- (99.3 %). Mean ALT values for both HPM+ and HPM- patients declined rapidly by week 1; ALT normalized by end of treatment in

95.4 % of HPM+ patients and in 95.5 % of HPM– patients. A higher rate of ALT normalization was observed in SVR12+ (97.1 % [165/170]) versus SVR12– (50 % [3/6]) patients, irrespective of HPM use. Among SVR12+ patients, HPM use had no impact on ALT normalization (HPM+: 97.1 %; HPM–: 97.0 %). OBV/PTV/r treatment was generally well-tolerated, with few treatment discontinuations due to TEAEs.

Conclusions: GIFT-I results indicate that HPM use in noncirrhotic Japanese patients during OBV/PTV/r treatment has limited value, because ALT normalizes with SVR12 achievement, regardless of HPM use. Confirmatory studies are needed.

O-037

Boceprevir is active for the treatment of genotype 6 HCV

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Background: Genotype (Gt) 6 hepatitis C (HCV) is prevalent in up to one-third of HCV-infected individuals in South-East Asia. Boceprevir (BOC), a HCV protease inhibitor, has antiviral activity against Gt6 HCV in vitro. We hypothesise that BOC is a potent inhibitor of Gt6 HCV replication in vivo, and in combination with pegylated interferon-alpha (pIFN) and ribavirin (RBV) will cure a high proportion of Gt6-infected patients.

Methods: This was an open-labeled pilot study of BOC in treatment-naïve Gt6-infected, non-cirrhotic individuals of Asian background, with the good response IFNL4 genotype (CC, rs12979860). Ten patients were randomly assigned to receive (A) BOC monotherapy (800 mg TID) for 5-days, followed by BOC plus pIFN/RBV for 24-weeks (n = 4); (B) BOC plus pIFN/RBV for 24-weeks (n = 3); or (C) pIFN/RBV for 48-weeks (n = 3). Participants randomised to BOC required a further 24-weeks of pIFN/RBV if HCV RNA detectable at week-4. Primary endpoint was early viral kinetics over the first 5-days of treatment.

Results: We enrolled ten patients-5/10(50 %) male, median age 37-years (IQR 33–49 years), median baseline serum HCV RNA level 6.321 log₁₀ IU/mL (IQR 5.942–6.993 log₁₀ IU/mL), and median liver stiffness measurement 5.1 kPa (IQR 4.6–7.1 kPa). Reduction in HCV RNA at d5 was observed in all 4-patients treated with BOC monotherapy lead-in (median reduction 2.505 log₁₀ IU/mL, Fig. 1). No differences in early viral kinetics (median reduction in HCV RNA at d5 = 2.505 vs. 1.741 vs. 1.681, p = 0.760) were measured between treatment arms. SVR12 90 % (9/10). Two patients withdrew due to intolerance (n = 1(Arm A) at w4; n = 1(Arm B) at w24).

Conclusions: BOC has antiviral efficacy for the treatment of Gt6 in vivo. The data suggest a potential role for BOC in combination DAA regimens for Gt6 HCV.

Primary endpoint: Early Viral Kinetics

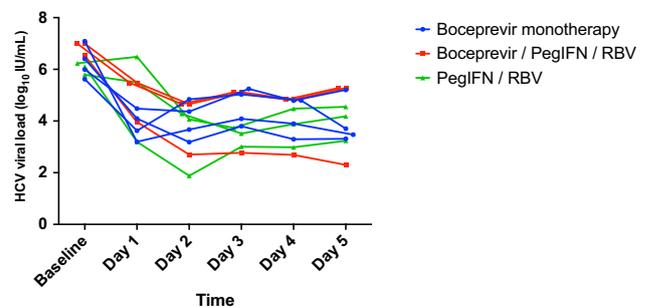


Fig. 1

O-038

GIFT-I: Ombitasvir/paritaprevir/ritonavir safety and efficacy in renally-impaired Japanese patients

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Background: To assess the safety/efficacy of coformulated ombitasvir/paritaprevir/ritonavir (OBV/PTV/r; PTV identified by AbbVie and Enanta) stratified by baseline (BL) estimated glomerular filtration rate adjusted for the Japanese population (eGFRj) <60 and ≥60 mL/min/1.73 m² in HCV genotype 1b-infected Japanese patients without cirrhosis or with compensated cirrhosis.

Methods: Non-cirrhotic treatment-naïve and IFN-experienced Japanese patients were randomized 2:1 to receive double-blind (DB) OBV/PTV/r for 12 weeks (Arm A) or DB PBO for 12 weeks followed by open-label (OL) OBV/PTV/r for 12 weeks (Arm B). Cirrhotic patients received 12 weeks of OL OBV/PTV/r. Sustained virologic response was assessed at post-treatment week 12 (SVR12). eGFRj <50 mL/min/1.73 m² was an exclusion criterion. Treatment-emergent adverse events (TEAE) from first dose through 30 days after last dose are presented for those who received ≥1 dose of active study drugs.

Results: SVR12 rates were similarly high in patients with eGFRj <60 mL/min/1.73 m² (65/70, 92.9 %) or ≥60 mL/min/1.73 m² (281/293, 95.9 %; Table). OBV/PTV/r treatment was generally well-tolerated in both groups with few treatment discontinuations due to TEAEs. One related grade 3 TEAE was reported in the ≥60 mL/min/1.73 m² group (hypotension), whereas 4 patients experienced related grade 3 TEAEs in the <60 mL/min/1.73 m² group included anuria, renal impairment, headache, nausea, vomiting, and pulmonary edema (1 report each). No grade 4 or 5 AEs were reported (Table).

Conclusions: The results of this study indicate that OBV/PTV/r therapy is effective and generally well-tolerated in Japanese patients with both a BL eGFR above 60 mL/min/1.73 m², as well as between 50 and <60 mL/min/1.73 m².

Efficacy	Baseline eGFR (mL/min/1.73 m ²)			
		<60	≥60	
SVR12, n/N % (95% CI)				
Total	65/70	92.9 (84.3, 96.9)	281/293	95.9 (93.0, 97.6)
Treatment-naïve	40/44	90.9 (78.8, 96.4)	167/172	97.1 (93.4, 98.8)
IFN-experienced	25/26	96.2 (81.1, 99.3)	114/121	94.2 (88.5, 97.2)
Safety, n (%)		N=70		N=293
Any TEAE		47 (67.1)		201 (68.6)
Any serious TEAE		4 (5.7)		8 (2.7)
Any grade 3, 4, or 5 TEAE*		8 (11.4)		8 (2.7)
Any TEAE leading to discontinuation of study drug		2 (2.9)		1 (0.3)
Any TEAE leading to interruption of study drug		1 (1.4)		0
Any fatal TEAE		0		0
Deaths [†]		0		2 (0.7)

CI: confidence interval; IFN: interferon; SVR12: sustained virological response at post-treatment week 12; TEAE: treatment-emergent adverse events.

*No events were higher than grade 3.

[†]Includes non treatment-emergent deaths.

O-039

Predictors of response to elbasvir/grazoprevir in HCV genotype 1-infected patients

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Background: In phase 2–3 trials, SVR12 was 95 % in GT1-infected patients receiving elbasvir 50 mg/grazoprevir 100 mg ± ribavirin. This analysis assessed predictors of response.

Methods: Two pooled datasets of GT1-infected patients were analyzed: treatment-naïve patients (TN; n = 801) and patients who previously failed peginterferon/ribavirin ± first-generation protease inhibitors (TE; n = 607). Univariate logistic regression was employed to assess potential associations between patient/disease characteristics and SVR12. Multivariable logistic regression models with forward selection were then applied to identify significant (P < 0.1) independent predictors of SVR12.

Results: In TN patients, age, sex, cirrhosis, HIV coinfection, baseline NS3 RAVs, and ribavirin did not impact SVR12. Among TN GT1a-infected patients, baseline HCV-RNA and NS5A RAVs conferring >5-fold elbasvir potency shift in vitro (NS5A >5 × RAVs) were predictors of SVR12. Baseline HCV-RNA was predictive only when NS5A >5 × RAVs were present (GT1a patients, 5.3 %). No predictors were identified among TN or TE GT1b-infected patients or GT1a patients with prior relapse. Among GT1a-infected patients with prior on-treatment failure, SVR12 was higher in females, noncirrhotics, those receiving ribavirin, and those with longer treatment durations. The most prevalent (>1 %) NS5A >5 × RAVs in TN and TE patients were L31M and Y93H. SVR12 was lower in TE patients with baseline NS5A >5 × RAVs treated for 12 weeks (8 % of TE GT1a patients).

Conclusions: Elbasvir/grazoprevir ± ribavirin is highly effective among GT1-infected patients. NS5A >5 × RAVs are uncommon;

their impact on SVR12 is limited to TN patients with high viral load and TE patients with prior on-treatment failure treated for 12 weeks.

O-040

GIFT-I: safety and efficacy of ombitasvir/paritaprevir/ritonavir in elderly Japanese patients

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Background: To assess the safety and efficacy of coformulated ombitasvir/paritaprevir/ritonavir (OBV/PTV/r; PTV identified by AbbVie and Enanta) in elderly (≥65 years) and non-elderly (<65 years) HCV genotype 1b-infected Japanese patients without cirrhosis or with compensated cirrhosis.

Methods: Non-cirrhotic patients aged 18–75 years in the GIFT-I phase 3 trial were randomized 2:1 to receive double-blind (DB) coformulated OBV/PTV/r 25/150/100 mg QD (Arm A) or PBO for 12 weeks followed by open-label (OL) OBV/PTV/r for 12 weeks (Arm B). Cirrhotic patients received 12 weeks of OL OBV/PTV/r. Sustained virological response was assessed at post-treatment week 12 (SVR12). Treatment emergent adverse events (TEAEs) from first dose through 30 days after last dose are presented for those who received ≥1 dose of active study drugs.

Results: 209 non-elderly patients (mean 55.1 years) and 154 elderly patients (mean 69.7 years) were evaluated. Overall SVR12 rates were high and similar between groups (Table). TEAEs were more frequent in elderly patients. Frequencies of specific TEAEs were generally similar between groups. The incidences of grade 3, 4 or 5/severe TEAEs, serious TEAEs, and TEAEs leading to study interruption or discontinuation were ≤6.5 %. No grade 4 or 5 TEAEs were reported (Table).

Conclusions: The results of this study indicate that OBV/PTV/r therapy is effective and well-tolerated in both elderly and non-elderly Japanese patients. The higher frequency of TEAEs in the elderly patients, as expected, may be due to a higher frequency and severity of comorbidities and/or due to a more frequent use of concomitant medications.

Efficacy	Baseline Age (years)			
	Non-Elderly (<65 years)		Elderly (≥65 years)	
SVR12, n/N % (95% CI)				
Total	202/209	96.7 (93.2, 98.4)	144/154	93.5 (88.5, 96.4)
Treatment-naïve	133/137	97.1 (92.7, 98.9)	74/79	93.7 (86.0, 97.3)
IFN-experienced	69/72	95.8 (88.5, 98.6)	70/75	93.3 (85.3, 97.1)
Safety, n (%)				
Any TEAE		131 (62.7)		117 (76.0)
Any serious TEAE		6 (2.9)		6 (3.9)
Any grade 3, 4, or 5 TEAE*		6 (2.9)		10 (6.5)
Any TEAE leading to discontinuation of study drug		0		3 (1.9)
Any TEAE leading to interruption of study drug		1 (0.5)		0
Any fatal TEAE		0		0
Deaths [†]		0		2 (1.3)

CI: confidence interval; IFN: interferon; SVR12: sustained virological response at post-treatment week 12;

TEAE: treatment-emergent adverse events.

*No events were higher than grade 3.

[†]Includes non treatment-emergent deaths.

O-041

Pan-oral direct acting antiviral agents for CHC GT1b Chinese-a real life experience

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Background and aims: Pan-oral direct-acting antiviral (DAAs) agents have recently been made available to treat patients with chronic hepatitis C (CHC) infection. We report the its efficacy, safety and cost-effectiveness in Chinese with CHC GT1b, in a real-life setting.

Methods: We evaluated 140 CHC (72 had cirrhosis-CPT A/B/C: 49/19/4) GT1b Chinese treated with Solvadi® plus Daklinza® (SOF-DCV, n = 46), Harvoni® (n = 48) or PEG-interferon/RBV (no CPT-C included, n = 46). Plasma HCV RNA concentration was measured at baseline and then 4-weekly till 24 weeks after end-of-treatment, by COBAS TaqMan assay. A decision analytic Markov model with a lifetime horizon and a third party perspective was developed by treatment history using real-life data.

Results: All patients (94/94) treated with SOF-DCV or Harvoni® had SVR12, as compared to only 29/46 (63 %) treated with PR48 had SVR24 (p < 0.001). Rate of HCV RNA decline was significantly slower in PR48 group than those treated with SOF-DCV or Harvoni (p < 0.001). Report of adverse events were lower in SOF-DCV or Harvoni® than PR48 treated patients. Compared to PR48, SOF-DCV and Harvoni® increased QALYs by 1.4 resulting ICERs of US\$84,272 and US\$37,755 respectively in treatment-naïve patients. At a willingness to pay of US\$22,782 (3 times GDP per capita in China), SOF-DCV or Harvoni® is not cost-effective in both treatment-naïve and experienced patients.

Conclusions: 12-weeks SOF-DCV or Harvoni® is highly effective for CHC GT1b (including cirrhotic) Chinese. However, due to the high cost, it is only cost-effective in less than half of the patients.

O-042

Change of AFP level according to the stage of liver disease during daclatasvir/asaunaprevir therapy

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Background: The decline of the AFP level is reported in the SVR achieved cases with IFN free therapy, but whether the AFP level becomes a surrogate marker of the liver carcinogenesis in the same way as IFN-based therapy is unknown. We compared the change of AFP level according to the liver stage of disease during DCV/ASV combination therapy.

Methods: 178 cases who accomplished 24-weeks DSV/ASV combination therapy consisting of chronic hepatitis (CH group: 85 case), liver cirrhosis (LC group: 60 cases) and cases having history of

hepatocellular carcinoma (HCC group: 33 cases) were enrolled. As for HCC group, we confirmed the absence of HCC recurrence by CT or by MRI before entry. AFP was measured every 4 weeks during therapy.

Results: The AFP level of baseline (BL) and end of therapy (EOT): CH group was 4.1 ng/ml (1.5–53.3) and 2.9 ng/ml (1.3–9.9), LC group was 8.3 ng/ml (1.3–634.9) and 4.4 ng/ml (1.1–23.8), and HCC group was 9.9 ng/ml (1.4–150.9) and 6.3 ng/ml (1.4–18.0). All group showed significant decrease. The ratio of high AFP level (>10 ng/ml) at BL and EOT was 11.8 and 0 % in CH group, 46.7 and 10 % in LC group, 48.5 and 21.2 % in HCC group. At EOT in LC and HCC group that thought to be high risk of the liver carcinogenesis, 10 to 20 % of high AFP level cases remained.

Conclusions: AFP decreased regardless of the stage of liver disease by DCV/ASV therapy, but a high AFP level cases remains in the LC and HCC group, and careful follow-up is necessary.

O-043

Therapy with daclatasvir and asunaprevir for dialysis patients with hepatitis C: case reports

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Interferon (IFN) monotherapy have been a standard antiviral therapy of the dialysis patients infected with genotype 1b hepatitis C virus. However, the efficacy of IFN monotherapy have been unsatisfactory for these patients. Although oral direct-acting antiviral agents (DAAs) have recently become available, some oral DAAs were contraindication for chronic renal impairment. Fortunately, daclatasvir and asunaprevir are both metabolized largely in the liver and are no contraindication to chronic renal failure. Thus, we tried this combination therapy for 4 dialysis patients infected with genotype 1b hepatitis C virus. Although one of patients had viral breakthrough and resultantly the combination therapy was discontinued in the middle of the treatment, three patients had SVR4. One patient experienced admission for heart failure and percutaneous coronary intervention due to concomitant ischemic disease. Heart failure was probably due to water overload and unlikely to bear a causal relationship to the combination therapy. In fact, the patient could continue to receive the combination therapy after the remission of heart failure. The other patients were well tolerated. Thus, the combination therapy was very useful strategy for dialysis patients with genotype 1b hepatitis C virus. We can show the results of SVR12 rate at the meeting.

O-044

Evaluation of pretreatment RAVs benefits for improved outcomes in daclatasvir/asunaprevir therapy

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Backgrounds: Interferon (IFN) free regimen for treatment of HCV infection is main stream in practice guidelines globally. In Japan, first IFN free regimen using daclatasvir (DCV: NS5A inhibitor) and asunaprevir (ASV: NS3-4 protease inhibitor) has been approved in 2014. The preceded Japanese phase 3 trial revealed SVR rate of 84.7 % in total cases. However, the existence of resistant anti-viral mutations (RAVs), especially Y93 mutations in NS5A region, had significantly negative effects in treatment outcomes. Thus, the measurements of RAVs before DCV/ASV treatments have been feasible in Japan. In this study, we have analyzed the influence of pre-treatment evaluation of RAVs in Yamagata Prefecture, northern-east region of Japan.

Methods: Total 629 patients with HCV infections have been evaluated RAVs before IFN free treatments until September 2015. Patients was recruited at community-based hospitals in Yamagata Hepatitis Network. Treatment effects was evaluated at 4 week (RVR), 12 week (EVR), and end of treatment (EOT).

Results: Among 629 patients, the prevalence of any RAVs was 22.9 % (n = 144), NS5A RAVs = 22.9 % and NS3 RAVs = 6.5 %. For detail, Y93 alone 15.7 % (n = 99), L31 alone 1.4 % (n = 9), D168 alone 0 %, Y93 + L31 3.3 % (n = 21), Y93 + D168: 1.4 % (n = 9), L31 + D168 1.0 % (n = 6), Y93 + L31 + D168 0.8 % (n = 5), was observed. After RAVs evaluation, 195 cases had been actually treated with DCV/ASV INF-free regimen. Among these treatment-initiated patients, only 6.1 % (n = 12) had RAV(s). Treatment effects (ITT) in available cases were: RAR 72.5 % (116/160), EVR 95.4 % (144/151), and EOT 93.1 % (81/87).

Conclusions: Evaluation of pretreatments RAVs seems to contribute for the improvement of treatments outcomes of DCV/ASV therapy.

related). The most common grade 3/4 laboratory abnormalities were aminotransferase elevations (more frequent among Japanese patients); however, all grade 3/4 laboratory abnormality occurred in <5 % of patients overall. Grade 3/4 total bilirubin elevations were reported in <1 % of patients. The DCV + ASV safety profile was similar in patients with or without cirrhosis.

Conclusion: DCV + ASV was generally well tolerated across global non-Asian patient populations and in Asian patients from Japan, mainland China, Korea, and Taiwan.

Patients, n (%)	Global ^a			Japan ^b				Asia ^c	Total ^{d,e}
	028 N=645	011 N=18	Total N=663	031 N=141	017 N=33	026 N=222	Total N=396	036 N=159	N=1218
Death	0	0	0	0	0	0	0	1 (<1)	1 (<0.1)
Serious AEs ^f	39 (6)	1 (6)	40 (6)	6 (4)	3 (9)	13 (6)	22 (6)	5 (3)	67 (6)
AEs leading to discontinuation	10 (2)	0	10 (2)	7 (5)	2 (9)	11 (5)	20 (5)	2 (1)	32 (3)
Grade 3/4 AEs	50 (8)	1 (6)	51 (8)	21 (15)	6 (21)	37 (17)	64 (16)	20 (13)	135 (11)
Emergent grade 3/4 laboratory abnormalities									
Hemoglobin <9 g/dL	1 (<1)	0	1 (<1)	0	0	7 (3)	7 (2)	1 (<1)	9 (1)
Platelets <50 × 10 ⁹ cells/L	11 (2)	0	11 (2)	0	0	4 (2)	4 (1)	16 (10)	31 (3)
Neutrophils <0.75 × 10 ⁹ cells/L	9 (1)	0	9 (1)	1 (<1)	0	1 (<1)	2 (<1)	1 (<1)	12 (1)
ALT >5 xULN	15 (2)	1 (6)	16 (2)	16 (11)	4 (9)	16 (7)	36 (9)	2 (1)	54 (4)
AST >5 xULN	12 (2)	1 (6)	13 (2)	7 (5)	3 (7)	12 (5)	22 (6)	3 (2)	38 (3)
Total bilirubin >2.5 xULN	3 (0.5)	0	3 (<1)	0	0	2 (1)	2 (<1)	1 (<1)	6 (<1)

AE, adverse events; ALT, alanine aminotransferase; AST, aspartate aminotransferase.
^a Includes studies: A1447028 [includes non-Asian, N=492; Asian, N=153], A1447011 [includes non-Asian, N=16; Asian, N=2]
^b Includes studies: A1447031, A1447017, A1447026 [all patients were Japanese]
^c Includes studies: A1447036 [includes mainland China, N=127; Korea, N=17; Taiwan, N=15].
^d AEs reported during rescue therapy (DCV plus ASV and pegIFN plus ribavirin) were not included in this analysis.
^e Only includes patient on the recommended DCV plus ASV dose (DCV 60 mg QD and the ASV 200 mg BID tablets or 100 mg BID softgel capsules)
^f Treatment-related serious AEs in 12 patients included pyrexia (n=5), hypochondriasis (n=1), aminotransferase elevation (n=5), bilirubin elevation (n=1), C-reactive protein increase (n=1), myasthenia gravis (n=1), atrial fibrillation (n=1) and hepatic enzyme increase (n=1); some patients experienced >1 serious AE.

O-045

Integrated Safety and Tolerability of DCV + ASV in Patients with Chronic HCV Genotype 1b Infection

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Background: The combination of daclatasvir (DCV) plus asunaprevir (ASV) has demonstrated high SVR rates and is generally well tolerated in clinical studies. This integrated analysis evaluated the safety profile of DCV (60 mg QD) and ASV (100 mg softgel capsule or 200 mg tablets BID) in genotype 1b (GT1b) infected patients enrolled in four phase 3 and two phase 2 clinical studies conducted globally, including Asia.

Methods: Integrated safety data from 1218 treatment-naïve or treatment-experienced patients were analyzed for adverse events (AEs), serious AEs, discontinuations due to AEs and grade 3/4 AEs and laboratory abnormalities reported on-treatment.

Results: Patients were 58 % female, median age was 58 years and 23 % had compensated cirrhosis. DCV + ASV was associated with infrequent serious AEs and discontinuations due to AEs (Table). Twelve patients reported treatment-related serious AEs. The most common AEs (any grade) were diarrhea, nausea, fatigue, and headache. One patient died due to coronary heart disease (not treatment-

O-046

Efficacy of daclatasvir/asunaprevir for chronic hepatitis C patients with renal insufficiency

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Purpose: The purpose of this study is to clarify the results of treatment in patients with renal insufficiency among those receiving daclatasvir/asunaprevir, which may not be theoretically influenced by the renal function.

Methods: Of 226 patients who received daclatasvir/asunaprevir in a multicenter cooperative study, we analyzed 136 in whom SVR8 could be evaluated. The protocol was approved by the Ethics Review Board of Nippon Medical School Chiba Hokusoh Hospital.

Results: Patients consisted of 136 (58 males). The median age was 72 years (range 46–85). Drug-resistance of the NS5A region at Y93 was detected in 7 patients. The eGFR levels were <50 in 22 patients, <30 in 7, and <10 in 5, respectively. The RVR, ETR, and SVR8 rates were 79.7, 89.6, and 84.6 % (115/136), respectively. Multivariate analysis showed that independent factors contributing to

achieving SVR8 were the absence of Y93 mutations, absence of previous simeprevir treatment, and an achieving of RVR. The RVR, ETR, and SVR8 rates in patients with an eGFR of <50 were 90.9, 100, and 100 % (22/22), respectively. In those with an eGFR of <30, they were 100 % (7/7). In particular, these rates were 100 % (5/5) even in those with an eGFR of <10, for whom dialysis was introduced. Furthermore, there were no adverse events, such as liver dysfunction or symptoms, characteristic of patients with renal insufficiency.

Conclusion: Daclatasvir/asunaprevir for chronic hepatitis C contributed to favorable virological responsiveness in patients with renal insufficiency. It can be safely performed even in those undergoing dialysis.

O-047

Efficacy and safety of daclatasvir and asunaprevir in elderly patients with HCV genotype 1b infection

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Background: Direct acting antivirals combination therapies now offer SVR rates greater than 90 % for treatment-naïve and experienced patients with genotypes 1 through 4. However, real clinical data for elderly patients are not fully evaluated. We investigated the efficacy and safety of daclatasvir (DCV) and asunaprevir (ASV) in elderly patients with HCV genotype 1b infection.

Methods: From September 2014 to September 2015, HCV genotype 1b infected patients over 75 years of age treated with daclatasvir 60 mg once daily plus asunaprevir 100 mg twice daily for 24 weeks were enrolled. No daclatasvir-resistant NS5A-L31 M or -Y93H/F variants was detected prior to the treatment. The primary endpoint was undetectable HCV RNA at 12 weeks post-treatment (sustained virologic response, SVR12) and the secondary endpoint was the frequency of serious adverse events (AEs) and discontinuations due to AEs.

Results: As of October 2015, 34 subjects (13 male and 21 female, mean age of 79.3, 9 patients were Child-Pugh A cirrhosis) had enrolled and SVR12 data were available in 21 patients. Among patients given DCV/ASV, 100 % (14/14) of naïve, 50 % (1/2) of breakthrough, and 100 % (5/5) of null responder achieved SVR12. 3 of 21 patients (14.3 %) experienced at least one AE, but most events were mild to moderate in severity. Three patients discontinued because of skin rash, abnormal liver function test, and viral breakthrough.

Conclusion: These findings suggest that DCV/ASV regimen is safe and effective in elderly patients.

O-048

Daclatasvir and asunaprevir for treatment of posttransplant hepatitis C virus cirrhosis

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Recurrence of hepatitis C virus (HCV) infection following liver transplantation (LT) is frequent and can lead to accelerated graft cirrhosis. Interferon-containing regimens are poorly tolerated in LT.

All-oral regimens have provided high response rates with fewer side effects in patients with recurrent HCV after LT. Daclatasvir in combination with asunaprevir is used in compensated cirrhotics but the data of use after LT are limited. A 55-year-old woman presented with HCV infection detected during screening. Genotype was 1b; she was unresponsive to pegylated-interferon and ribavirin. On follow-up, she decompensated, and a living-donor liver graft was transplanted. One month after LT, she developed an acute flare. She was under mycophenolate mofetil (MMF) and steroid treatment; MMF was discontinued. She could not be given any antiviral therapy and ALT levels spontaneously decreased. One year after LT, a control liver biopsy revealed cirrhosis (Ishak's fibrosis score of 5/6). The patient was given daclatasvir 60 mg per day and asunaprevir 100 mg, two times in a day, 24 weeks. She was closely followed-up and the dose of immunosuppressive drug (steroid) was tapered since no drug-drug interaction data were available. She developed sustained virological response. In post-transplant settings, daclatasvir + asunaprevir has been used for a patient with severe cholestatic hepatitis. Our case represents the first time use of daclatasvir with asunaprevir in post-transplant cirrhosis. Daclatasvir combined with asunaprevir seems to be an option for post transplant HCV recurrence in patients including compensated cirrhotics.

O-049

An efficacy and safety of daclatasvir/asunaprevir therapy for HCV-positive kidney transplantation

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Background/aims: In most of cases, hepatitis C virus (HCV) re-infection occurs in kidney transplant (KT) recipients and is an important cause of morbidity and mortality. Interferon (IFN)-based therapies induce severe rejection of the allograft and generally contraindicated. While the novel direct-acting antiviral drugs (DAAs) represent a promising treatment option for KT recipients, there are few reports of the efficacy and safety of DAAs under immunosuppression. Here, we report seven cases treated with DAAs after KT and evaluated an efficacy and safety.

Methods: The seven patients were treated with NS5A and NS3 protease-targeted DAA (daclatasvir, DCV and asunaprevir, ASV) therapy for 24 weeks after KT.

Results: The median age was 55 (49–71) years, and there were five males. The acquired HCV was serological type 1 and the L31 and Y93 wild-type strain of the NS5A polymorphisms. The median HCV RNA level was 6.5 (5.0–7.0) log₁₀ IU/mL. The immunosuppressants prescribed were corticosteroids/mammalian target of rapamycin, tacrolimus, and mycophenolate mofetil, or azathioprine. Treatment was started at 5 (0.5–35) years after KT. The tacrolimus concentration was maintained and no substantial dose adjustment was required. Treatment was ongoing in three cases and five patients became HCV RNA-negative within 2 months. One case was dropped out due to mild fever and renal impairment, however, still achieved sustained virologic response. The other cases showed no severe adverse events in liver or renal function.

Conclusions: An IFN-free regimen of DCV/ASV therapy was a safe and effective in HCV-positive KT recipients, even under immunosuppressive conditions.

O-050

DCV + ASV in IFN (\pm RBV)-ineligible/intolerant Asian patients with chronic HCV genotype-1b infection

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Background: The efficacy/safety of daclatasvir (pan-genotypic NS5A inhibitor) plus asunaprevir (NS3 protease inhibitor) in interferon (\pm ribavirin)-ineligible/intolerant patients with chronic HCV genotype-1b infection from mainland China, Korea and Taiwan was investigated in a phase 3, open-label study.

Methods: Patients received daclatasvir 60 mg (tablet) once daily plus asunaprevir 100 mg (soft capsule) twice daily for 24 weeks. The primary endpoint was sustained virologic response at post-treatment Week 24 (SVR24).

Results: This study treated 159 patients from mainland China (80 %), Korea (11 %) and Taiwan (9 %), including patients with cirrhosis (33 %), *IL28B* non-CC genotypes (40 %), and aged ≥ 70 years (4 %). SVR24 was achieved by 91 % of patients (100 % concordance with SVR12) and was similarly high in all subgroups, e.g. cirrhotic patients (90 %), and in patients from mainland China (91 %), Korea (94 %) and Taiwan (87 %). SVR24 was higher in patients without baseline NS5A (L31 M/Y93H) resistance-associated variants (RAVs) ($n = 137/139$ [99 %]), regardless of the presence (98 %) or absence (99 %) of cirrhosis, and lower in patients with baseline NS5A RAVs ($n = 8/19$ [42 %]). All serious adverse events (AEs) ($n = 5/159$ [3.1 %]), grade 4 laboratory abnormalities ($n = 3/159$ [1.9 %]) and deaths ($n = 1/159$ [0.6 %]) that occurred on-treatment were unrelated to the study drugs; two patients discontinued due to AEs. Treatment was generally well tolerated regardless of cirrhosis status.

Conclusion: Daclatasvir plus asunaprevir achieved a high SVR24 rate of 91 %, rising to 99 % in patients without baseline NS5A RAVs, and was generally well tolerated in cirrhotic and non-cirrhotic interferon (\pm ribavirin)-ineligible/intolerant patients with HCV genotype-1b infection from mainland China, Korea and Taiwan.

O-051

Hepatitis C viruses with Y93H mutation in NS5A region are susceptible to interferon-based therapy

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Background & aims: Direct acting antivirals (DAAs) dramatically improve the outcome of chronic hepatitis C treatment in Japan. But the resistance-associated variants (RAVs) of hepatitis C viruses (HCV) attenuate the efficacy of DAAs. We aimed to characterize the susceptibility of RAVs to interferon-based therapy.

Methods: Forty genotype 1b patients with detectable Y93H variant in NS5A region at baseline were enrolled. All of them were treated by a combination of pegylated interferon, ribavirin, and simeprevir (NS3/4A inhibitor). The longitudinal changes of RAVs were determined deep sequencing during therapy and at breakthrough or relapse.

Results: In 29 cases with Y93H proportion over 5 % at baseline, the proportion of Y93H variant was decreased from 52.7 % (5.8–97.4 %) at baseline to 29.7 % (0.16–98.3 %) ($p = 0.023$) within 7 days of initiation of treatment by deep sequencing. The patients with Y93H variant over 90 % in proportion maintain the proportion during therapy. The proportion of Y93H variant decreased in 17 of 22 cases in whom the Y93H proportion detected within 5–90 %, and markedly reduced more than 10 % in 14 of 22 cases (63.6 %). In 4 cases with breakthrough/relapse, the proportion of Y93H RAV decreased during therapy, but recovered to baseline 3 months after discontinuation of treatment. HCV RNA reduction was significantly greater for Y93H RAV (-3.65 ± 1.3 logIU/mL/day) than the Y93wild type (-3.35 ± 1.0 logIU/mL/day) ($p < 0.001$). There was no increase of Y93H proportion during and after therapy. In 11 cases with Y93H proportion under 2 % at baseline.

Conclusion: Y93H RAV is more susceptible than the Y93wild type to interferon-ribavirin-simeprevir combination therapy.

O-052

NS5A RAVs in DAA-naive HCV genotype 1b Japanese patients were associated with patients' background

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Aim: Although direct-acting antivirals (DAAs) may potentially be useful to treat hepatitis C virus (HCV)-infected patients, the presence of resistance-associated variants (RAVs) may reduce the efficacy of such therapy. The aim of the present study was to characterize naturally occurring L31 and Y93 variants in the nonstructural 5A (NS5A) region.

Methods: Direct sequencing of NS5A region was performed to identify the RAVs in 432 HCV genotype 1b infected patients. The

IL28B rs8099917 polymorphism was genotyped by TaqMan SNP genotyping assay.

Results: L31-resistance mutations were found in 17 patients (3.9 %), and Y93 in 48 (11.1 %); 4 patients had double mutations. The prevalences of the L28, R30, Q54, P58, Q62 and A92 variants were 1.6, 8.1, 47.9, 9.3, 8.1 and 6.7 %, respectively. Viral loads were significantly higher in resistant patients (with L31 and/or Y93 mutations) than in sensitive patients (wild-type at both L31 and Y93) (6.5 vs. 6.2 log IU/mL, $p = 0.02$). Daclatasvir-resistant substitutions were more common in IL28B major genotype than in non-major genotypes (16 vs 9 %, $p = 0.04$). Interferon-based therapy may be one of the anti-viral options for patients with NS5A RAVs.

Conclusion: NS5A RAVs were detected in Japanese patients infected with HCV genotype 1b, without previous DAA therapy. In addition to DAA resistant, NS5A RAVs were associated with patients' background.

O-053

Invader assay for HCV NS5A RAVs is excellent for the prediction of outcome of DCV/ASV treatment

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HCV NS5A inhibitor daclatasvir (DCV) plus HCV NS3/4A inhibitor asunaprevir (ASV) treatment for 24 weeks could lead to higher sustained virologic response (SVR) in patients with HCV GT-1b if they have no HCV NS5A resistance-associated variants (RAVs). We performed screening for HCV RAVs by invader assay and selected the patients for DCV/ASV treatment. In total 100 patients, 10 (10 %) and 24 (24 %) were with RAVs at L31 and Y93H, respectively. Total 33 patients (33 %) had RAVs at L31 and/or Y93H. Finally we selected total 54 patients without Y93H (54 %) (69.2 years; male, 35.2 %; treatment-naïve, 31.5 %; cirrhosis, 61.1 %) and treated them by DCV/ASV. Total 46 patients remained negative HCV RNA and 43 of these 46 patients had already achieved end of treatment response. Only 1 of 54 patients (1.9 %) experienced virologic break-through (VBT). Total 7 patients stopped the treatment due to adverse events, and 2 of 7 had positive HCV RNA and 5 of 7 had negative HCV RNA. At the present, DCV/ASV led to virologic response in 51 patients (94.4 %). Invader assay for HCV NS5A RAVs is useful for the selection of the HCV GT-1b patients for DCV/ASV treatment.

O-054

DAA therapies for treatment-emergent variants of HCV in humanized liver chimeric mice

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Background/aim: DAAs treatment failures induce the emergence of resistance-associated variants (RAVs). In this study, we evaluated the efficacy of retreatment by DAAs for HCV with treatment-emergent RAVs.

Methods/results: Thymidine kinase transgenic NOG mice with humanized liver were inoculated with serum obtained from 2 patients under institutional IRB approval. One is a patient who had failed to achieve sustained virologic response by asunaprevir (ASV)/daclatasvir (DCV) and the other is a patient who had not experienced DAA therapy. After inoculation, they developed persistent HCV infection with HCV RNA levels up to 5–7 log IU/ml in serum. Population and deep sequencing revealed that mice inoculated with the former's serum carried NS3/4A D168V, NS5A L31V plus Y93H mutant virus while mice with the latter's carried wild-type virus at these positions. HCV-infected mice were treated with ASV/DCV or ledipasvir (LDV)/GS-558093 (nucleotide NS5B polymerase inhibitor) for 4 weeks. HCV RNA levels of wild-type HCV rapidly declined and sustained undetectable levels in both treatment. In contrast, HCV RNA levels of mutant virus reduced 1–2 log IU/mL or 2–3 log IU/mL from baseline by ASV/DCV or LDV/GS-558093, respectively, but did not decrease to undetectable levels. These mice were re-treated by GS-558093/ telaprevir, of which resistance profile differs from ASV. Their serum HCV RNA levels rapidly decreased to undetectable levels.

Conclusion: The mutant virus emerging upon the treatment failure of ASV/DCV is relatively resistant to LDV/GS-558093. Other DAA regimens that do not share cross-resistance may be a choice of re-treatment for such case.

O-055

The clinical profiles of patients with L31 and Y93 aa variants in NS5A region of hepatitis C virus.

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Background and aims: It is known that resistance-associated variants (RAVs) of NS5A inhibitors naturally occur at positions L31 and Y93 amino acids. Since the clinical profiles are still unclear, we investigated the relationship between RAVs and clinical factors.

Patients and methods: Patients with genotype 1 and who were treatment naïve for NS5A inhibitors were selected. We measured the Y93 positions in 1225 patients and L31 positions in 1067 patients by direct sequencing. We also measured the Y93 positions in 1067 patients by the Cycleave PCR method. Among them, in 691 patients introduced by daclatasvir and asunaprevir (DCV/ASV) therapy, various clinical factors were also analyzed to find the association with RAVs.

Results: In the 1225 patients, the Y93H variant was detected in 187 patients (15.3 %). Among these 187 patients, 166 patients were measured by the Cycleave PCR method: 25.3 % (42/166) of the patients had 100 % of the Y93H variant. Patients introduced by DCV/

ASV therapy with the Y93H variant had a significantly higher HCV RNA level ($p < 0.0001$), lower platelet count ($p = 0.0008$), an older age ($p = 0.0005$), and presence of liver cirrhosis ($p = 0.0044$) than patients with the Y93 wild type. The related factors with completion of SVR12 in DCV/ASV therapy are Y93 wild type ($p < 0.0001$), lower HCV RNA level ($p = 0.0091$).

Conclusion: HCV RNA levels are closely related to RAVs and the effectiveness of DCV/ASV therapy. Careful attention should be paid on treatment with DCV/ASV, and the interaction among RAVs and clinical features should be analyzed.

O-056

HCV patients treated with IFN-free regimens experience improvement of patient-reported outcomes

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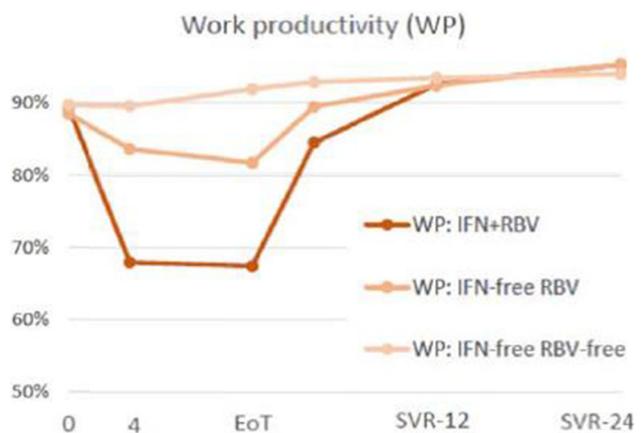
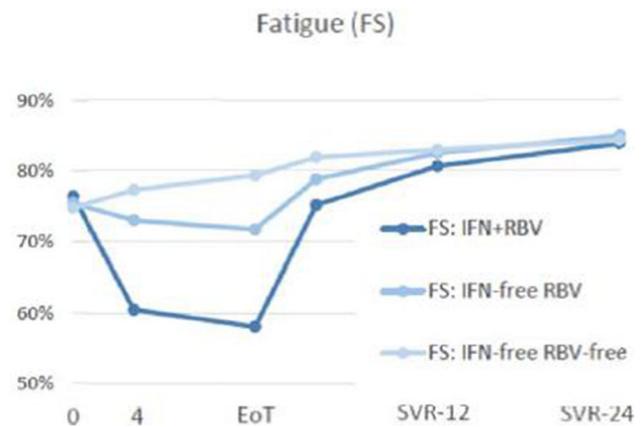
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Background: Due to substantial side effects, interferon (IFN) and ribavirin (RBV)-containing regimens have negative impact on patient reported outcomes (PROs). New regimens free of IFN and RBV have minimal side effects and a potentially superior PRO profile. Our aim was to use a large PRO database and compare the impact of different treatment regimens on PROs and work productivity.

Methods: We used PRO data from 8 multinational clinical trials of different regimens used to treat CHC. During these trials, four PRO instruments (SF-36, CLDQ-HCV, FACIT-F, WPAI:SHP) were administered at baseline, during, and up to 24 weeks post-treatment. Treatment regimens were classified as IFN + RBV-containing, IFN-free RBV-containing (SOF + RBV or LDV/SOF + RBV), and IFN-free RBV-free (LDV/SOF).

Results: We included 3425 CHC patients with PRO and clinical data: 62.8 % male, 62.2 % treatment-naïve, 18.1 % cirrhotics, 72.9 % GT1, 5.6 % GT2, 20.3 % GT3, and 546 receiving IFN + -SOF + RBV, 1721 SOF + RBV, 1158 LDV/SOF. No baseline PRO differences between the groups was found. On-treatment decrements in PRO scores were noted up to -23.6 and -7.0 % for IFN + RBV + SOF and SOF + RBV, respectively, in contrast to an improvement up to $+11.6$ % in LDV/SOF (all $p < 0.0001$). In multivariate analysis, IFN- and RBV-use were independently associated with PRO impairment. Patients who achieved SVR-12 experienced PRO improvements regardless of regimen, and the magnitude of this improvement was increasing throughout 24 weeks of follow-up ($p < 0.05$).

Conclusions: The use of IFN- and RBV-free regimens for treating CHC is associated with better patients' experience, PRO scores and work productivity during treatment. Patients with SVR experience significant and sustainable PRO improvements.



O-057

Efficacy of a shortened 12-week SMV + PR regimen in HCV GT1 patients according to *IL28B* genotype

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Introduction: A Phase 3, open-label study of simeprevir (SMV) (150 mg QD) plus peg-interferon/ribavirin (PR) was conducted in treatment-naïve patients with chronic HCV GT1 infection and METAVIR F0–F2 fibrosis, with treatment duration (12 or >12 weeks) determined according to early viral kinetics. High SVR rates with triple therapy have been observed in patients with *IL28B* CC genotype (more common in Asian than Caucasian patients). We investigated the effect on efficacy of shortening overall SMV + PR treatment to 12 weeks in patients with Week 2 virologic response, according to *IL28B* genotype (CC or non-CC).

Methods: 163 patients (40 with *IL28B* CC genotype) with HCV GT1 participated in the study (Table). Patients with HCV RNA <25 IU/mL detectable/undetectable at Week 2, and <25 IU/mL undetectable at Week 4 and 8 (Roche COBAS® Taqman® assay, LLOQ: 25 IU/mL, LOD: 15 IU/mL) stopped all treatment at Week 12; patients not meeting these criteria continued PR to Week 24.

Results: Overall, 123/163 (75 %) patients, including 32/40 (80 %) with *IL28B* CC genotype, were eligible for 12-week treatment. 38/40 (95 %) of all CC patients, including 30/32 (94 %) receiving 12-week treatment, achieved SVR12; lower SVR12 rates were seen in patients with *IL28B* non-CC genotype (Figure). The two *IL28B* CC patients not achieving SVR12 experienced viral relapse. The SMV + PR safety profile across all participants was consistent with previous studies.

Conclusions: In treatment-naïve patients with HCV GT1 and *IL28B* CC genotype, an algorithm based on Week 2 virologic response may help identify patients able to shorten overall treatment duration to 12 weeks, without reducing efficacy.

O-058

Inhibitory effects of telaprevir on orexigenic ghrelin secretion in patients with HCV infection

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Aim: Telaprevir is a potent protease inhibitor. However, treatment with telaprevir plus Peg-IFN and ribavirin frequently induces anorexia. Ghrelin is an orexigenic gut hormone. In the present investigation, the mechanisms of anorexia in patients with telaprevir-based therapy, were studied in relation to plasma level of acyl ghrelin, an active type of ghrelin.

Methods: (1) Telaprevir- and simeprevir- based triple therapies were respectively performed in 9 and 11 patients with HCV genotype 1b. Appetite and food intake were estimated by the visual analogue scale (VAS) score, and plasma samples after an overnight fast for the measurement of acyl and desacyl ghrelin levels were collected before, and 1 or 2, and 8 days after the initiation of the therapy. (2) Fasted rats were administered telaprevir or vehicle. Plasma acyl and desacyl ghrelin levels were measured 2 or 4 h after the injection.

Results: (1) In telaprevir group, VAS scores of appetite and food intake and plasma acyl ghrelin level significantly decreased on day 1 or 2 compared with those before the therapy. The decreases were attenuated on day 8. There were no significant differences in desacyl ghrelin level. In simeprevir group, there were no differences in VAS scores and both ghrelin levels. (2) Acyl ghrelin level at 4 h but not

2 h, and food intake within 24 h in rats treated with telaprevir were significantly decreased compared with controls.

Conclusions: It was suggested that anorexia early induced by telaprevir-based therapy was at least partially mediated through inhibition of acyl ghrelin secretion.

O-059

Chronic hepatitis C with restoration of innate-immunity with n-IFN-beta followed by simeprevir

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Complications associated with chronic HCV infection may be prevented by viral eradication. Response rates with direct-acting -antivirals are generally lower in chronic hepatitis C (CHC) patients who have failed to respond to previous interferon (IFN) treatment. HCV persistence results from inefficient innate and adaptive immune responses. The resolution of a HCV infection may restore impairments in innate and adaptive immunities. Our previous study suggested that induction treatment (IT) with natural (n)-IFN-beta restored innate immune responses, as indicated by the up-regulation of IL-12, and IL-15 and down-regulation of CXCL-8. Persistent virologic clearance and the restoration of innate immune responses with Simeprevir plus Pegylated (Peg)-IFN-alpha/ribavirin (RBV) were more likely to result in a sustained virologic response (SVR). On the basis of these findings, we conducted to treat a CHC patient with genotype 1b, a high viral load, a prior null response to IFN treatments and CHC with advanced fibrosis using IT with n-IFN-beta followed Simeprevir plus Peg-IFN-alpha-2b/RBV. Anemia and thrombocytopenia were managed without discontinuation of the treatment. This is the first case of difficult-to-treat CHC with genotype 1b, a high viral load, null response to previous IFN treatment and advanced hepatic fibrosis that was successfully treated with IT using n-IFN-beta, which induced the viral clearance of HCV RNA, followed by Simeprevir plus Peg-IFN-alpha-2b/RBV. Persistent viral clearance linked to restoration of innate immune responses, SVR and sustained biochemical response were achieved 24 weeks after cessation of the treatment. IT with n-IFN-beta followed by Simeprevir plus Peg-IFN-alpha-2b/RBV was effective and tolerated well.

O-060

A phase 2 dose finding study for P1101 + ribavirin in patients with chronic HCV genotype 1 infection

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Aim: Ropeginterferon alfa-2b (P1101) is a novel long-acting mono-pegylated recombinant proline-interferon alfa-2b conjugated to a 40kD branched polyethylene glycol chain at its N-terminus. The study aimed to find the optimal dose of P1101 + ribavirin for the treatment of patients with chronic HCV genotype 1 infection.

Methods: 106 treatment naive patients with chronic HCV genotype 1 infection were randomized to one of 4 treatments: subcutaneous (SC) weekly PEGASYS 180 mcg (control), P1101 180 mcg, 270 mcg or biweekly P1101 450 mcg, plus daily oral ribavirin 1000 mg (<75 kg) or 1200 mg (≥75 kg). All patients who have ≥2-log drop in serum HCV RNA at Week 12 received 48-week regimen and 24-week follow-up.

Results: The study is ongoing. Among patients who have completed the study, SVR rates are 77 % (20/26), 67 % (20/30), 93 % (13/14), and 58 % (7/12) for weekly PEGASYS, P1101 180 mcg, 270 mcg, or biweekly P1101 450 mcg, respectively. Safety and tolerability of P1101 are comparable to PEGASYS, even at the highest dose. P1101 270 mcg group has the best safety profile.

Conclusions: P1101 is effective and safe at all doses. There is a strong suggestion that P1101 270 mcg SC weekly + daily oral ribavirin may be more efficacious and safer than PEGASYS 180 mcg SC weekly + daily oral ribavirin for the treatment of Asian patients with chronic HCV genotype 1 infection.

O-061

Identification of SNPs in HLA class II gene related to disease progression of chronic hepatitis C

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Background: Recent genome-wide studies have demonstrated that HLA class II gene may play an important role in viral hepatitis. We studied genetic polymorphism and RNA expression of HLA class II genes in HCV-related liver diseases.

Methods: The study was performed in groups consisting of 23 patients with HCV-related liver disease (11 of persistent normal ALT: group A and 12 of advanced liver disease: group B) and 26 patients without HCV infection (group C). In PBMC samples, RNA expression of HLA class II genes (HLA-DPA1, DPB1, DQA1, DQB1 and DRB1) was analyzed by real-time RT-PCR. Furthermore, 22 single nucleotide polymorphisms (SNPs) in HLA class II gene and two SNPs in IL28B gene were genotyped by genetic analyzer (GENECUBE®).

Results: In expression analysis, only DPB1 level was significantly different. Mean expression level of DPB1 gene in group C was 160.0, group A 233.8 and group B 465.0 ($p < 0.01$). Of 24 SNPs, allele

frequencies were statistically different in two SNPs (rs2071025 and rs3116996) between groups A and B ($p < 0.01$). In rs2071025, TT genotype was frequently detected in advanced liver disease (group B) and expression level was significantly higher than the other genotypes (449.2 vs 312.9, $p < 0.01$). In rs3116996, TA or TT (non AA) genotype was frequently detected in advanced liver disease (group B) and expression level was significantly higher than genotype AA (457.1 vs 220.9, $p < 0.01$).

Conclusions: Genotyping and expression analysis in HLA class II gene revealed that two SNPs of HLA-DPB1 (rs2071025 and rs3116996) were significantly correlated to progression of HCV-related liver diseases.

O-062

A phase 2 dose finding study for P1101 + ribavirin in patients with chronic HCV genotype 2 infection

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Aim: Ropeginterferon alfa-2b (P1101) is a novel long-acting mono-pegylated recombinant proline-interferon alfa-2b with a 40kD branched polyethylene glycol chain conjugated predominantly at its N-terminus. The study aimed to find the optimal dose of P1101 by comparing sustained virologic response (SVR), safety and tolerability across treatment groups in patients with chronic HCV genotype 2 infection.

Methods: Eighty-six treatment naive patients with chronic HCV genotype 2 infection were randomized to receive subcutaneous injection of weekly peginterferon alfa-2a at 180 mcg, or biweekly P1101 at 270 mcg, 360 mcg and 450 mcg, plus daily oral ribavirin 1000 mg (≤75 kg) or 1200 mg (>75 kg). Patients with rapid virologic response (RVR) received 16-week regimen while others without RVR received 24-week regimen. Each patient received 24-week post-treatment follow-up.

Results: Among patients completing the treatment and 24-week post-treatment follow-up, SVR rates were 95 % (21/22), 78 % (18/23), 90 % (18/20), and 67 % (12/18) with peginterferon alfa-2a, and P1101 270, 360, 450 mcg, respectively. Incidence rates of adverse events during the treatment were 0.424, 0.260, 0.393, and 0.352 per patient week exposure in peginterferon alfa-2a, and P1101 270, 360, 450 mcg groups, respectively.

Conclusions: P1101 has fewer adverse events than peginterferon alfa-2a. The antiviral efficacy of biweekly P1101 at 360 mcg plus daily

oral ribavirin is comparable to that of weekly peginterferon alfa-2a at 180 mcg plus daily oral ribavirin. These data prompt us to conduct a phase III clinical trial in patients with chronic HCV genotype 2 infection.

O-063

Switching PEG-RBV to DAAs for Chinese with chronic hepatitis C GT1b

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Background and aim: Early discontinuation from pegylated interferon/ribavirin (PR) therapy is not uncommon in patients with chronic hepatitis C (CHC) infection, due to treatment-intolerance. We examine the efficacy and safety of switching PR-treated CHC Chinese to pan-oral direct-acting antiviral (DAAs) in a real-life setting.

Methods: 129 consecutive CHC GT1b Chinese who were initiated with PR and had completed early virologic response (cEVR, undetectable serum HCV RNA level at week 12 but not by week 4), were studied. Twenty (16 %) discontinued PR therapy, due to PR-intolerance and were switched to 4 (n = 10) or 8 (n = 10) weeks of Harvoni® (ledipasvir/sofosbuvir), at patient's discretion. Plasma HCV RNA concentration was measured at baseline and then 4-weekly till 24 weeks after end-of-treatment, by COBAS TaqMan assay. A decision analytic model was developed and a cycle of 4-week was applied in the first 52 weeks and yearly cycle was applied afterwards. Outcome measures include discounted cost (in 2014 US\$), quality-adjusted-life-years, and incremental cost-effectiveness ratio (ICER).

Results: All CHC patients treated with Harvoni had SVR12 as compared to 52/109 who continued PR therapy (100 % Vs 48 %, p < 0.0001). Report of adverse events is significantly lower in those who switched to Harvoni. Compared to PR48, PR12 + 4 weeks Harvoni and PR12 + 8 weeks Harvoni gained 1.021 and 1.019 QALYs, with a reduced cost of US\$1,206 and US\$1004 per person.

Conclusions: In CHC GT1b patients with complete early virologic response, switching to 4–8 weeks Harvoni is safe, effective and cost-saving.

O-064

The antiviral therapy based on HCV NS5A resistant mutations in hepatitis C virus genotype 1b.

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Objectives: Virologic failure of Daclatasvir (DCV) and asunaprevir (ASV) combination therapy was associated with baseline NS5A resistant associated variants (RAVs) such as L31M and Y93H mutation and screening for the presence of RAVs at baseline is

recommended. The sustained virologic response (SVR) for patients with NS5A RAVs treated with DCV and ASV therapy is reported as 43 %. In those patients, alternative treatment such as Simeprevir (SMV) triple therapy would be needed. The aim is to assess the rate of sustained virologic response in SMV plus IFN therapy and DCV and ASV therapy according to baseline NS5A RAVs.

Methods: Twenty two patients with NS5A RAVs who received SMV plus pegylated-IFN and ribavirin combination therapy and sixty three patients without NS5A RAVs who received ASV/DCV therapy were selected for this study.

Results: Of the 63 patients, 58 (92.1 %) achieved SVR by DCV and ASV therapy. Of the 22 patients, 14 (63.6 %) achieved SVR by SMV plus IFN therapy. Achievement of SVR occurred in patients with IL28B TT allele (92.3 %) and those with TG and GG alleles (16.6 %). There were significant differences in SVR by IL28B genotype in patients with SMV plus IFN therapy (p = 0.0029).

Conclusions: SMV plus IFN therapy could improve SVR rate for patients with NS5A RAVs who are eligible for IFN-based therapy. The screening for the presence of NS5A RAVs at baseline and IL28B genotype are useful for planning the strategy for HCV treatment.

O-065

Detecting NS5A-Y93H mutations and analyzing its clinical significance in HCV-1b patients.

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Backgrounds: It recently became apparent that NS5A mutations (L31M and Y93H) are frequently found in DAA-naïve HCV-1b patients, and those patients are at risk to develop clinical resistance to NS5A inhibitors. Therefore, accurate NS5A mutation detection system is indispensable. On the other hand, it is unknown if there exist clinical characteristics in those with mutations.

Methods: (1) In 110 DAA-naïve patients, NS5A mutation was determined with deep sequencing, direct sequencing and invader assay. (2) In 427 DAA-naïve patients, NS5A mutation was determined by direct sequencing and clinical characteristics specific to mutant patients were investigated.

Results: (1) Deep sequencing analysis revealed that the L31M and Y93H mutations were present in 13/110 (11.8 %) and 34/110 (30.9 %) patients, respectively. When the results of deep sequencing was used as the gold standard of Y93H mutations, sensitivity/specificity for Y93H detection in direct sequencing and in invader assay was 72 %/98 % and 85 %/100 %, respectively. (2) IL28B TT (OR 3.0, p = 0.03), female (OR 2.3, p = 0.03) and non-HCC concurrence (OR 3.2, p = 0.03) were associated with patients with NS5A-Y93H. In multivariate analysis for HCC occurrence among factors of age (>65 years old), gender (male), liver stiffness (>12 kPa) and NS5A-Y93 (wild), all these four factors including NS5A-Y93 (wild) were extracted as independent affecting the occurrence of HCC.

Conclusions: Recently-developed invader assay was superior to direct sequencing in the detection of Y93H mutation, however, further improved system is still needed. Those Y93H patients showed specific clinical characteristics, indicating that clinician should determine candidates for NS5A inhibitor-included regimens after careful consideration of clinical backgrounds.

O-066

Deep sequencing of variants resistant to protease inhibitor therapy in HCV-1b hepatitis

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Background: To clarify the evolution of viral quasiespecies and the origin of resistant mutation induced by DAAs, we performed deep sequencing study for patients with NS3-4A protease inhibitors (PIs) combined with pegylated-interferon (PEG-IFN)/ribavirin (RBV).

Methods: Pyrosequencing and phylogenetic analysis were performed in 34 genotype-1b HCV patients receiving telaprevir (TVR)/PEG-IFN/RBV therapy and 26 genotype-1b HCV patients receiving simeprevir (SMV)/PEG-IFN/RBV therapy.

Results: In TVR/PEG-IFN/RBV therapy, 26 patients (76.5 %) achieved a sustained viral response (SVR) while 8 patients did not (non-SVR, 23.5 %). When the complexity of the quasiespecies was expressed as mutation frequency or Shannon entropy, a significant decrease in the IFNL3 (rs8099917) TT group and a marginal decrease in the SVR group were found soon (12 h) after the introduction of treatment, whereas there was no decrease in the non-SVR group and no significant decrease in mutation frequency in the IFNL3 TG/GG group. In the analysis of viral quasiespecies composition in non-SVR patients, major populations greatly changed accompanied by the appearance of resistance and the compositions were unlikely to return to the pretreatment composition even after the end of therapy. In SMV/PEG-IFN/RBV therapy, 22 patients (84.6 %) achieved SVR while 4 patients did not (non-SVR, 15.4 %). Clinically SMV-resistant variants were observed in all 4 non-SVR patients. All TVR and SMV clinically resistant mutations were suspected to have been acquired by mutations through phylogenetic analysis.

Conclusions: The associations among treatment response, changes in viral complexity in the early stage of treatment, changes in the quasiespecies composition, and origin of PIs-resistant variants were elucidated.

O-067

Simple non-invasive scores in predicting significant liver related events in chronic hepatitis C

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Introduction: We compared nineteen simple non-invasive fibrosis scores (NIFS), derived from routine blood tests, for predicting response to antiviral treatment (AVT) and significant liver related adverse events (SLRE) in patients with chronic hepatitis C (CHC).

Methods: 1605 patients (from 2002 to 2014) underwent liver biopsy (LB, Scheuer-staging) and received AVT (pegylated interferon and ribavirin). Nineteen simple NIFS were calculated from routine blood tests, prior to AVT. AUROCs were calculated for each of these NIFS for predicting non-response to AVT and development of SLRE (defined as development of any event requiring intervention; decompensation and hepatocellular carcinoma[HCC]) on follow-up.

Results: Median age 42 (range 14–73) years, (85 % males), predominantly genotype-4 (65 %) and genotype-1 (11 %). After AVT, there were 1089 (67.8 %) responders, 482 (30 %) non responders and 34 (2.1 %) relapsers. After median follow-up of 6580.5 patient-years post-treatment; 52 (3.2 %) had decompensation (bleed-9, ascites-39, jaundice-22, hepatic encephalopathy-7, spontaneous bacterial peritonitis-10, hepatorenal syndrome-4), 11 (0.7 %) had HCC and 62 (3.9 %) had SLRE. The predictive accuracy of NIFS and LB for non-response to AVT was low (Table-1). FIB-4, FibroQ and King score showed high accuracy for predicting adverse events. For predicting decompensation, HCC and SLRE, FibroQ (0.881), King score (0.905) and FibroQ (0.877) respectively had the highest AUROC. New NIFS, red cell distribution width (RDW) to platelet count ratio had relatively high AUROC for predicting both decompensation and SLRE.

Conclusions: Some simple pre-treatment NIFS showed high accuracy (more than that of LB) for predicting development of decompensation, HCC and SLRE after antiviral treatment. Application of these simple scores can improve assessment of long term liver prognosis in CHC.

Table 1 Role of non-invasive fibrosis scores in predicting treatment non response, decompensation, HCC and SLRE

	Treatment non-response*	Decompensation*	HCC*	SLRE*
AST-platelet ratio index (APRI)	0.519(0.440-0.599),0.041	0.538(0.063-0.789),0.120	0.800(0.660-0.940),0.071	0.535(0.299-0.771),0.119
Fibrosis-4 (FIB-4)	0.983(0.504-0.562),0.040	0.854(0.738-0.956),0.041	0.884(0.827-0.942),0.029	0.847(0.765-0.942),0.039
Lok score	0.518(0.438-0.598),0.041	0.803(0.858-0.993),0.127	0.529(0.289-0.789),0.123	0.801(0.552-0.999),0.129
GUCl score	0.521(0.442-0.601),0.041	0.804(0.062-0.779),0.144	0.843(0.717-0.970),0.065	0.600(0.318-0.882),0.139
Fibro alpha	0.551(0.472-0.631),0.041	0.544(0.362-0.999),0.146	0.615(0.296-0.835),0.112	0.644(0.280-0.923),0.144
Modified APRI	0.587(0.508-0.666),0.040	0.615(0.101-0.784),0.132	0.521(0.367-0.927),0.117	0.608(0.349-0.967),0.129
King score	0.575(0.508-0.664),0.041	0.641(0.016-0.888),0.150	0.903(0.858-0.954),0.025	0.635(0.342-0.923),0.149
AST-ALT ratio	0.533(0.495-0.654),0.041	0.671(0.405-0.999),0.137	0.543(0.365-0.923),0.094	0.671(0.405-0.923),0.132
Pohl score	0.506 (0.429-0.584), 0.04	0.619 (0.392-0.836),0.128	0.683 (0.558-0.990),0.012	0.609 (0.282-0.836),0.116
Fibro-index	0.526 (0.448-0.604),0.039	0.678 (0.445-0.873), 0.114	0.841 (0.673-0.990), 0.085	0.679 (0.475-0.883), 0.104
Age platelet index	0.640 (0.568-0.712),0.037	0.816 (0.663-0.948), 0.078	0.613 (0.549-0.977), 0.033	0.806 (0.663-0.958), 0.078
Cirrhosis discrimination score	0.615 (0.542-0.668), 0.037	0.804 (0.610-0.988), 0.089	0.658 (0.521-0.995), 0.019	0.814 (0.640-0.998), 0.089
Globulin albumin ratio	0.529 (0.450-0.608), 0.040	0.452 (0.225-0.661), 0.110	0.515(0.000-1.000), 0.281	0.452 (0.235-0.669), 0.111
Mean platelet volume	0.542 (0.464-0.620), 0.040	0.648 (0.478-0.756), 0.067	0.455 (0.285-0.625), 0.087	0.628 (0.489-0.786), 0.077
RDW to platelet count ratio	0.598 (0.522-0.674), 0.039	0.839 (0.742-0.959), 0.060	0.710 (0.677-0.943), 0.017	0.856 (0.740-0.971), 0.059
Fibrosis index	0.555 (0.433-0.613),0.040	0.767(529-0.873),0.079	0.761(0.676-0.999),0.054	0.764(0.610-0.917),0.091
Fibrosis cirrhosis index	0.531 (0.517-0.673),0.041	0.646(0.603-0.998),0.143	0.604(0.365-0.926),0.022	0.644(0.365-0.923),0.141
Globulin platelet index	0.500 (0.451-0.611),0.041	0.500(297-0.800),0.146	0.498(0.266-0.725),0.117	0.500(0.214-0.785),0.122
FibroQ	0.585(0.476-0.634),0.040	0.881(0.730-0.950),0.076	0.807(0.723-0.891),0.043	0.877(0.727-0.968),0.065
Cirrhosis(StageF4 fibrosis)#	0.485(0.405-0.564),0.041	0.480(205-0.756),0.141	0.558(0.308-0.809),0.128	0.484(202-0.751), 0.039

* AUROC (confidence interval), standard error, # Liver biopsy

O-068

Fibroscan is more predictive of hepatocarcinogenesis in chronic hepatitis C than serum markers

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Background: Hepatocellular carcinoma is one of the major problems in patients with chronic hepatitis C (CHC). We previously reported that Fibroscan (FS) is a useful non-invasive tool to predict hepatocarcinogenesis (Hepatology 2008). However, the comparisons with other serum markers of liver fibrosis such as platelet count, aminotransferase platelet ratio index (APRI) score and FIB-4 remained to be elucidated.

Methods: In this retrospective study, we enrolled 866 patients with CHC examined using FS between December 2004 and June 2005, evaluated occurrence of hepatocarcinogenesis until December 2014 using the Kaplan-Meier procedures and compared with the log-rank test. We compared predictability of FS for hepatocarcinogenesis with that of platelet count, APRI or Fib-4 using Harrell's c-index.

Results: Of the 866 patients, 398 (46.0 %) patients were male and the median age was 64.0 years. The median (with 25th–75th percentiles) of FS, platelets, APRI and FIB-4 were 8.3 (5.6–14.6) kPa, 160 (110–203) × 1000/μL, 0.74 (0.39–1.42), 2.77 (1.70–4.74). Five-year cumulative incidences of hepatocarcinogenesis in patients stratified by quartiles of FS, platelets, APRI and FIB-4 were 2.1 %/1.5 %/11.4 %/40.4 % (P < 0.0001), 32.2 %/14.2 %/7.1 %/2.0 % (P < 0.0001), 1.6 %/3.8 %/19.0 %/31.1 % (P < 0.0001), 0 %/4.5 %/16.5 %/34.4 % (P < 0.0001), respectively. A c-index based on established risk factors for hepatocarcinogenesis was 0.850 and it was increased to 0.868 (+0.018), 0.853 (+0.003), 0.856 (+0.006) and 0.855 (+0.005) by adding FS, platelets, APRI and FIB-4, respectively.

Conclusions: FS is more predictive of hepatocarcinogenesis in patients with CHC than other serum markers of liver fibrosis.

O-069

Vaccination study of cell culture-generated hepatitis C virus particles in non-human primate model

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About 150 million people globally have chronic hepatitis C infection. Although novel direct-acting antivirals have increased the number of patients who respond to treatment, it is still important to develop the vaccine to prevent the infection of this virus. In this study, we aimed to establish the prophylactic vaccine of HCV by exploiting the cell culture-generated HCV (HCVcc), and validated the efficiency of neutralizing antibody induction in non-human primate model. Non-human primate; common marmosets were immunized with purified J6/JFH-1 chimeric virus (genotype 2a/2a) subcutaneously and intramuscularly. In combination, two kinds of adjuvants were used, classical adjuvant; Alum or recently developed potent adjuvant; K3-SPG (schizophyllan conjugated CpG). Serum samples were collected from vaccinated common marmosets, and antibody titers to HCV envelope and core proteins were measured. The neutralizing efficiencies of induced antibodies were also assessed. By immunization with HCVcc and K3-SPG, efficient induction of HCV specific antibodies was detected. Induced antibodies reacted to E2 glycoproteins of J6 (genotype 2a) and TH (genotype 1a) strains. These antibodies

also recognized the core protein of strains of genotypes 1a, 1b and 2a. Purified IgG from these serum samples indicated the neutralizing activity in vitro HCV infection system with J6/JFH-1 (genotype 2a/2a) and TH/JFH-1 (genotype 1b/2a) chimeric viruses. In conclusion, the combination of HCVcc and K3-SPG worked as the potent and safety vaccine of HCV in non-human primate model. This promising vaccine enabled the efficient induction of cross-neutralizing antibodies against strains of multiple genotypes.

O-070

Effect of splenectomy on immune cell state in hepatitis C virus related cirrhotic patients

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Objective: To investigate the immunological changes after splenectomy in hepatitis C virus cirrhotic patients with portal hypertension, and to evaluate the immunological function of hypertensive spleen.

Methods: From December 2011 to December 2013, 12 HCV cirrhotic patients with portal hypertension underwent splenectomy were included in this study. We evaluated natural killer cell and natural killer T cell activity, T-lymphocyte subsets such as CD4+, CD8+ and CD4+/CD8+ ratio by flow cytometry in peripheral blood before and 2, 6 weeks after splenectomy. The control group consisted of 12 health subjects.

Results: Compared with control group, the ratio of CD3-CD56 + CD16 + NK and the subsets of CD56dimNK cell were significantly decreased in cirrhotic patients. The activity of CD3-CD56 + CD16 + NK cell and CD56dimNK cell were significantly enhanced in 2 and 6 weeks post-splenectomy. The activity of CD3 + CD56 + NKT cell also was significantly enhanced in 2 weeks post-splenectomy. Compared with control group, the ratio of CD4+ T cells to all lymphocytes was significantly increased, but the ratio of CD8+ T cells was significantly decreased in cirrhotic patients. The ratio of CD4+ T cells to all lymphocytes were decreased and the ratio of CD8+ T cells to all lymphocytes were increased in 2 and 6 weeks post-splenectomy, resulting in a significantly decrease in the CD4+/CD8+ ratio.

Conclusion: Hypertensive spleen may play a negative role in immune regulation in patients with HCV cirrhosis. Splenectomy may improve cellular immune function in HCV cirrhotic patients.

O-071

Cytokine levels associated with risk for hepatocellular carcinoma among patients with HCV infection

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Background: The aim of this study is to screening cytokines with the predictability for hepatocellular carcinoma (HCC) among patients with hepatitis C virus (HCV) infection.

Methods: This study included 636 subjects seropositive for antibodies against HCV and seronegative for HBsAg in R.E.V.E.A.L.-HCV cohort. The participants were enrolled in 1991 and follow-up till the end of 2011 by computerized data linkage with the national cancer registration system and the national death certification database. At study entry, the questionnaires and blood samples were collected. The Cytokine assay by Merck Millipore was utilized to explore concentration of 38 cytokines in study subjects. The Bonferroni's corrections were used for multiple comparisons. The Cox's proportional hazards models were utilized to obtain the adjusted hazard ratios (HRs) with 95 % confidence intervals of the cytokines and the associated risk for HCC.

Results: Among the subjects there were 110 newly-diagnosed HCC cases after 10,836 person-years of follow-up, giving the HCC incidence rate of 1015 per 100,000 person-years. Each cytokine was categorized as low, medium, and high levels by the first and third tertile of the study subjects. Significant differences were found in 22 cytokines by comparing HCC and non-HCC cases ($p < 0.001$). There were 10 cytokines significantly associated with HCC risk after adjustment of age, gender, serum levels of AST, ALT, and HCV RNA ($p < 0.002$). The adjusted HRs ranged from 1.95 (1.09–3.46) to 10.71 (4.39–26.08).

Conclusions: Cytokine levels might be associated with risk for HCC among patients with HCV infection after long-term follow-up.

O-072

High prevalence of comorbidities and contraindicated medications in CHC patients in Japan

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Aim: To determine the prevalence of comorbidities and drug-drug interactions (DDIs) in CHC patients in Japan.

Methods: Patients were identified using the ICD10 code for CHC in the Japanese Medical Data Center (MDC) database (04/2008-08/2014). Prescriptions were categorized as either red (contraindicated) or amber (additional monitoring/dose reduction required) for DDI potential with at least one currently licensed direct-acting antiviral (DAA).

Results: 92,294 patients were identified, average age was 68 and 52 % male. 82 % of patients had one or more comorbidity; the number with 6 + comorbidities increased with age from 2 % of patients aged 18–34 to 17 % for patients 75+. The most common were hypertension (44 %), chronic gastritis (33 %) and gastro-oesophageal reflux disease (32 %). 74 % were treated with amber DDIs and 26 % were on red. Polypharmacy increased with age, from 43 % for 18–34 to 82 % for 75+ (amber) and from 13 to 29 % for 18–34 year olds and 75+ respectively (red). Only 8.2 % of patients were treated for CHC. Of these, 81 % had a potential DDI, increasing from 61 % for 18–34 years to 90 % for 75+.

Conclusions: We observed significant co-morbidity and co-prescribing with DDI potential in CHC patients in Japan. Few patients received CHC treatment, indicating a large unmet need in Japan. With the treatment shift from interferon to DAA's, more patients may receive treatment. Hence, the high proportion of co-medications contraindicated to all DAA's vs. only some suggests careful selection of the DAA regimen is required. Treating patients at a younger age would also reduce the risk of DDI.

O-073

Evaluation of B cell activating factor during the interferon based or free therapy in CH-C patients

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Background: Infection of HCV is associated with B cell abnormalities, such as lymphoproliferative disorders and autoimmune diseases. These disorders are thought to result from abnormal activation of B cells induced by direct stimulation with HCV and/or the trans-acting factors, such as B cell activating cytokines; BAFF/BLyS and APRIL. Serum level of BAFF was reported to be higher in patients with chronic hepatitis C (CH-C). In this study, we monitored serum BAFF and APRIL levels in CH-C patients who were treated with the interferon (IFN) free therapy and the combination therapy of telaprevir (TVR) or simeprevir (SMV), pegylated IFN and ribavirin.

Methods: 73 patients were enrolled in this study. Eighteen patients were treated with the IFN free therapy, 35 patients with TVR therapy, and 20 patients with SMV therapy. Serum levels of BAFF and APRIL were monitored by the enzyme immune assay in 4 time-points during the therapy (before, 1, 16 weeks, and 8 weeks after therapy).

Results: Serum levels of BAFF and APRIL were increased from 1 to 16 weeks after the beginning of therapy and decreased at 8 weeks after therapy in the IFN based therapy. While levels of BAFF and APRIL were stationary during the IFN free therapy statistically, indicating that risk of adverse events due to the immune disorders was less frequently.

Conclusion: The B cell activating cytokines were produced through the IFN based therapy. Resulting possible induction of abnormal B cells activation, The IFN free therapies may prevent these adverse events.

O-074

Predictors of adherence to treatment of chronic hepatitis C (CHC)

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Background: A number of patient- and treatment-related factors affect treatment adherence. Our aim was to assess the impact of

clinical and PRO factors on adherence to different treatment regimens in CHC.

Methods: Clinical and PRO data (SF-36, CLDQ-HCV, FACIT-F, WPAI:SHP) from 13 multinational clinical trials of anti-HCV treatment were used. Treatment non-adherence was defined as <80 % of doses taken for any of the drugs used.

Results: 4825 CH-C patients (64.7 % treatment-naive, 19.3 % cirrhotic, 65.1 % genotype 1) were included. Regimens included IFN + RBV-containing [PEG-IFN + RBV ± SOF: 14 %], IFN-free RBV-containing [RBV + sofosbuvir (SOF) ± ledipasvir (LDV): 52 %] and IFN-free/RBV-free [LDV/SOF: 31 %]. The adherence rates for the drugs were: LDV/SOF 97.5 %, SOF 95.1 %, IFN 86.4 %, RBV 85.1 %. Prior to treatment, non-adherent patients were more likely unemployed (48 vs. 39 %), depressed (32 vs. 26 %) ($p < 0.05$) and experienced with more PRO impairment (up to -5.3 % lower PROs, $p < 0.0001$). During treatment, non-adherent patients further experienced lower PRO scores (up to -11.2 %, $p < 0.001$) with larger decrements from baseline scores (up to -12.9 vs. -5.4 % in adherent patients, $p < 0.0001$). Patients non-adherent to IFN-containing regimens showed the lowest PRO scores and largest PRO decrements by the end of treatment (up to -28.9 %, $p < 0.0001$) versus modest PRO decrements in patients receiving RBV-containing regimens (up to -8.3 %, $p < 0.0001$). No significant declines in PRO scores were noted in patients non-adherent to LDV/SOF (all $p > 0.05$). Longer treatment duration was associated with non-adherence ($p < 0.05$).

Conclusions: The use of PEG-IFN, duration of treatment, as well as baseline and on-treatment PRO scores are the most important predictors of non-adherence to anti-viral regimens.

O-075

Hepatitis C treatment in a primary care clinic in the high HCV burden setting in Karachi, Pakistan

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Background: Burden of hepatitis C infection in Pakistan is among the highest in the world. Though national HCV prevalence reported to be 4, 9 %, pockets with higher prevalence exist in urban communities in Karachi. INF-free treatment for chronic HCV infection (CHC) could allow scale up, simplification and decentralization of treatment to these communities. We present data from the first months of CHC programme in the community clinic in Machar Colony, Karachi, Pakistan.

Methods: Patients were screened for HCV according to WHO 2014 guidelines. CHC was confirmed by presence of HCV RNA. APRI index was used to prioritize initiation of treatment. Eligible patients were treated with 12 or 24 weeks of Sofosbuvir and weight-based Ribavirin. HCV viral load was measured at baseline, end of treatment, and 12 weeks after treatment completion. Data was collected under routine programme conditions.

Results: 2539 patients who attended the MSF clinic between March and October 2015 were tested for HCV antibody. 719 (28 %) were positive. Of these, 646 (89 %) were found to have CHC. 176 (27 %) of patients with CHC had APRI >1.0. 111 HCV genotype results were available: 99 (89 %) had GT 3, 9 (8 %) GT1, and 3 (3 %) GT2. 111 patients initiated treatment. To date, 6 patients completed treatment and all had negative HCV RNA at completion.

Conclusions: Interim results suggest feasibility of the CHC treatment delivery at the primary care within the affected community, using simplified diagnostic and treatment algorithms.

O-076

Inhibition of HBsAg on HCV replication by increasing NKG2D expression on NK cells in CHB patients.

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Aim: By constructing the co-cultured system of NK cells and JFH-Huh7 HCV replication cells, we will investigate the effect of HBsAg on HCV replication and its natural immune mechanism.

Methods: After isolated from peripheral blood of 31 chronic hepatitis B (CHB) patients and 16 healthy individual, NK cells were co-cultured with JFH-Huh7 HCV replication cells respectively. Then, exogenous HBsAg was added into the co-cultured system. At 72 h time point, HCV RNA levels in co-cultured supernatant were detected by real-time quantitative PCR (TaqMan COBAS). NKG2D and NKp46 expression on NK cells were detected by flow cytometry before and after stimulated by HBsAg.

Results: There was no significant difference in HCV RNA levels of supernatant between single JFH-Huh7 cells and JFH-Huh7 cells stimulated by HBsAg (4.64 ± 0.71 vs. 4.92 ± 0.41 , $P = 0.600$). Normal NK cells can reduce the supernatant HCV RNA levels in the co-cultured system significantly (4.92 ± 0.41 vs. 3.85 ± 0.91 , $P = 0.006$), and NK cells of CHB patients cannot (4.92 ± 0.41 vs. 4.26 ± 0.86 , $P = 0.060$). NK cells of CHB patients can significantly reduce HCV RNA level (4.26 ± 0.86 vs. 3.57 ± 1.37 , $P = 0.022$) after HBsAg was added into co-cultured system, and NK cells of healthy controls cannot (4.09 ± 0.67 vs. 3.85 ± 0.91 , $P = 0.224$). In CHB patients, compared with that of untreated NK cells, the expression of NKG2D on exogenous HBsAg stimulated NK cells increased significantly (24.43 ± 19.10 vs. 58.62 ± 15.51 , $P = 0.000$), and however, the expression of NKp46 on NK cells was not significantly changed (24.43 ± 19.10 vs. 15.11 ± 12.53 , $P = 0.185$). The basic expression level of NKG2D on NK cells was no difference between CHB patients and healthy controls (24.43 ± 19.10 vs. 15.11 ± 12.53 , $P = 0.185$), and the expression level of NKG2D on NK cells of CHB patients was significantly higher than that of healthy controls (58.62 ± 15.51 vs. 41.68 ± 6.55 , $P = 0.000$) after NK cells were treated by HBsAg.

Conclusion: HBsAg can inhibit HCV replication in vitro, which may be mediated by activating NKG2D expression of NK cells in CHB patients.

O-077

Analysis of hepatitis C virus genotypes from previously discrepant diagnostic results

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Background: Hepatitis C Virus (HCV) genotypes are one of the factors that can affect the course of infection and outcome to antiviral regimens. Nowadays many HCV genotyping systems have been developed. Among them, serological test is a common method in Japan. However, some samples have been noticed as discrepant results from genotyping methods based on PCR and/or sequencing. In this study, we analyzed the difference between HCV serotype and genotype, and performed sequence analysis to uncover the mechanisms.

Methods: Total 14 serum samples were determined by serotyping using type-specific peptide in NS4 region. Genotyping was done by genotype-specific PCR in 5' untranslated region (UTR) of HCV. These two different methods have been shown discrepant results. Therefore these samples were analyzed again by sequencing and cloning of HCV sequences in the Core and NS4 regions based on nested-PCR of those regions. To investigate the possibility of a mixed infection, up to five different clones were sequenced and analyzed using Genetyx version 10 software.

Results: Nine out of 14 samples have been shown discrepant results, 1 have been determined as mixed type and the rest 4 have not been identified by serotyping or genotyping. Phylogenetic tree analysis assigned same genotypes between Core and NS4 regions in all samples. Furthermore, these results were concordant with the genotyping results from 5'UTR. There were a few amino acid variations when compared with reference peptides.

Conclusion: Multiple genotyping methods will be needed if there is a discrepancy to confirm a definite genotype for successful treatment.

O-078

Acute hepatitis C (AHC)

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Background: AHC is rarely diagnosed due to its predominantly asymptomatic course. Symptomatic AHC occurs in 20 % of patients with HCV infection and has higher likelihood of spontaneous viral clearance (SVC). PCR is a reliable test for diagnosing AHC due to delaying of Anti-HCV production up to 12 weeks.

Aim: To present description and course of AHC.

Methods: Among 82 patients with HC, AHC was diagnosed in 27 (33 %) of them last year. The mean age of patients with AHC was 40.8 ± 0.9 (20–62) and 65 %-men. In this study we prospectively assessed the clinical, biochemical, serological and virological parameters. The average time from exposure to manifestations of hepatitis was 67 days (20–115).

Results: All 27 patients had jaundice with elevated serum bilirubin 120 (40–200 $\mu\text{mol/L}$) and elevated ALT levels 1450 U/l (500–2400). The risk factors were: medical procedures 59 %, drug using 22.7 %, sexual exposure 8 %, unknown 10.3 %. In 7 (26 %) patients with AHC the diagnosis was confirmed by HCV RNA, without anti-HCV. The period of disease manifestations lasted 20–60 days, in 17 (62 %) patients with rapid decline in ALT. The following period of 6 months revealed SVC in 10 (36 %) patients with rapid decline in ALT, 8 of them—20 to 40 years old, 7 were females.

Conclusions: Symptomatic AHC was mostly associated with medical procedures and had high rates of spontaneous resolution-PCR was reliable test for diagnosing AHC. Rapid decline in ALT was the main risk factor for SVC as well as female sex and young age.

O-079

Ferrokinesics, calcium, vitamin D and red blood cell indices in treatment of chronic hepatitis C

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Aim: To correlate pre-treatment serum iron indices, RBC indices [mean platelet volume (MPV), red cell distribution width (RDW) and RDW/platelet count index (RPI)], calcium and vitamin-D levels with cirrhosis on liver biopsy (LB) and antiviral treatment response (AVT) in chronic hepatitis C (CHC).

Methods: We analyzed data of 1602 patients with CHC who underwent LB (Scheuer staging) and received AVT (pegylated interferon and ribavirin). Pre-treatment baseline parameters including serum iron indices, calcium, vitamin-D levels and RBC indices were correlated with LB fibrosis stages and response to AVT.

Results: The median age was 41 (range 14–64) years (1365 males), the most common genotype was genotype-4 (65.6 %). LB results showed stage-0 fibrosis in 1.9 %, stage-1 in 32.9 %, stage-2 in 39.5 %, stage-3 in 19 %, and stage-4 (cirrhosis) in 6.6 % of the patients. On univariate analysis (UVA) high iron ($p < 0.001$), high ferritin ($p < 0.001$), high transferrin saturation ($p < 0.001$), high MPV ($p = 0.01$), high RPI ($p < 0.001$) correlated with cirrhosis on LB. On UVA, high serum iron levels ($p < 0.001$), high serum ferritin ($p < 0.001$), low vitamin-D ($p = 0.036$) and high RPI (0.02) correlated with non-response to AVT. On multivariate analysis (Table-1) only AST, albumin and platelet count were independent predictors of cirrhosis and age, GGT, AST and platelet count were independent predictors of non-response to AVT.

Conclusions: Iron indices and RPI correlated with cirrhosis on LB and non-response to AVT on univariate analysis. However, only AST, albumin and platelet count were independent predictors of cirrhosis. Age, GGT, AST and platelet count were independent predictors of non-response to AVT.

Table-1

Independent predictors of cirrhosis on liver biopsy and antiviral treatment non-response

1	Independent predictors of cirrhosis on liver biopsy	Albumin: AOR* = 0.842, CI* = 0.742-0.915, $p = 0.001$ AST: AOR = 0.980, CI = 0.974-0.989, $p = 0.001$ Platelet count: AOR = 1.015, CI = 1.008-1.022, $p = 0.001$
	Predicting model for cirrhosis	$8.5 - 0.2(\text{albumin, g/dL}) + 0.01(\text{AST, IU/L}) - 0.02(\text{platelet count, } 10^9/\text{dL})$ AUROC [®] 0.867, CI - 0.838-0.890, SE [^] = 0.022
2	Independent predictors of treatment non-response	Age: AOR = 1.036, CI = 1.007-1.065, $p = 0.01$ ALT: AOR = 0.990, CI = 0.984-0.996, $p = 0.002$ GGT: AOR = 1.012, CI = 1.007-1.017, $p < 0.001$ platelet count: AOR = 0.989, CI = 0.984-0.995, $p < 0.001$
	Predicting model for treatment non-response	$0.072 + 0.035(\text{age, years}) - 0.01(\text{ALT, IU/L}) + 0.01(\text{GGT, IU/L}) - 0.01(\text{platelet count, } 10^9/\text{dL})$ AUROC 0.748 (95% CI = 0.708-0.789), SE = 0.022

*Adjusted odds ratio, * confidence interval, [®]Area under receiver operating characteristic curve,

[^] Standard error

O-080

Decreased ISGs in B cells of CH-C during IFN free therapy suggest eradication of HCV from B cells

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Background: Viral infection is regulated by the innate immunity, which consists of such host factors as interferon (IFN) and IFN stimulated genes (ISGs). HCV abolishes them, leading high levels of ISGs gene expression in liver of some patients. We reported HCV replication and/or association with B cells, and presence of HCV in B cells correlated with immunologic disorders and resistance to the IFN based therapy. However, HCV replication in B cells is hard to demonstrate due to low levels of viral replication. In this study, we monitored levels of ISGs in B cells of HCV infected patients during the interferon free therapy and investigated effects of viral eradication for the ISG levels. **Methods:** Forty seven patients infected with HCV, who achieved SVR, were enrolled in this study [DAA-IFN therapy: 30, IFN free therapy: 17]. The B cells were isolated by the auto-MACS at before, during (one, 16 weeks) and post therapy (8 weeks). The mRNA expression of ISGs (Mx1, OAS1, OAS2, ISGF3 and IFITM) was analyzed by the real-time RT-PCR.

Results: The mRNA levels of all the ISGs statistically increased 1 week after the beginning of the DAA-IFN therapy, while those of all the ISGs except ISGF3 significantly decreased in B cells through the IFN free therapy.

Conclusion: The DAA-IFN therapy leads B cells to the strong antiviral circumstance via higher ISGs level. The IFN free therapy directly eradicates HCV, leading endogenous ISGs to downregulate. These results indirectly suggest that HCV replicates in B cells.

O-081

Hepatitis C virus infection reduces chemo-sensitivity of the hepatocellular carcinoma cells

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Background and aim: Hepatitis C virus (HCV) infection causes the transformation of hepatocytes. However, the effect of HCV infection on the chemo-sensitivity of hepatocellular carcinoma (HCC) cells is unclear. In this study, we investigate the involvement of HCV infection in the regulation of chemo-sensitivity of HCC cells.

Methods: HCV-infected or non-infected control cells were treated with anti-cancer drugs including epirubicin. Cell viability and the protein expression of apoptotic markers of treated cells were evaluated. β -galactosidase staining of the cells were performed.

Results: Cell viability of HCV-infected cells was significantly higher than that of control cells after anti-cancer drug treatment. The expressions of apoptotic markers in epirubicin-treated HCV-infected cells

were significantly less than those in control cells. Proliferation assay showed that the growth of HCV-infected cells was slower than that of control cells. Cell cycle analysis demonstrated the cell cycle arrest at G0/G1-phase in HCV-infected cells. Population of β -galactosidase positive cells was significantly higher in HCV-infected cells than that in control cells. These results suggest that HCV infection causes cellular senescence, and induces cell cycle arrest or anti-apoptotic effect. HCV eradication by the treatment with interferon- α reduced the population of β -galactosidase positive cells, and also improved the chemo-sensitivity of HCC cells, suggesting that cellular senescence caused by HCV infection could be reversible after viral clearance.

Conclusion: HCV infection reduces chemo-sensitivity of HCC cells. Cellular senescence is considered to be one of the causes of this anti-apoptotic effect. HCV treatment should be applied for HCC patients.

O-082

Bivalent vaccine antigen for Japanese encephalitis virus and Hepatitis C virus

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Directly acting antivirals have recently become available for the treatment of hepatitis C virus (HCV) infection, but there is no prophylactic vaccine for HCV. In this study, we took advantage of the properties of Japanese encephalitis virus (JEV) to develop HCV vaccine. We explored sites in the JEV envelope protein that is exposed on the surface of the viral particle and is tolerant of foreign epitope insertion by expressing JEV subviral particles (SVP). Among the 11 sites we examined in the JEV E protein, 3 permitted insertion of the HCV E2 neutralization epitope recognized by the AP33 or HCV-1 antibodies. JEV SVP containing HCV neutralization epitope (SVP-E2) at one of these sites, or at all 3 sites, was expressed in 293T cells, and was purified from culture supernatant by gel chromatography. JEV SVP displaying a foreign epitope on the surface was confirmed by fluorescence correlation spectroscopy analysis using a specific antibody. Sera from mice immunized with SVP-E2 showed reduced infection with JEV and trans-complemented HCV particles (HCVtcp) derived from genotypes 1b, 2a and 3a, whereas sera from mice immunized with KLH-conjugated synthetic peptides of the neutralization epitope did not show any neutralizing activity. Furthermore, sera from mice immunized with SVP-E2 neutralized HCVtcp with N415 K mutation in E2, which is the escape mutant from HCV-1 antibody. Thus, a JEV particle-based platform harboring foreign epitopes on the surface could lead to the development of a bivalent vaccine against JEV and other pathogens.

O-083

Decreased titers of IgG-AMA are associated with biochemical response to UDCA treatment in PBC

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Background and aims: As the cornerstone of medicine therapy for primary biliary cirrhosis (PBC), UDCA exerts the efficacy in biochemical and histological improvement. Although AMAs represent the disease-specific serological hallmark of PBC, current data regarding the change pattern of AMAs during treatment course are scarce. Here, we assessed the influence of UDCA treatment on titers of AMA subtypes. **Methods:** Twenty-eight patients with PBC, including 17 patients from a clinical trial of UDCA therapy for 24 weeks, and 20 healthy controls (HCs) were enrolled. Titers of serum IgA-, IgM-, and IgG-AMA and serum levels of BAFF were detected by ELISA. The frequency of circulating plasmablasts was analyzed by FACS. CD38, IgA, IgM, and IgG expressing cells were detected by immunohistochemistry staining of liver tissues.

Results: Significantly decrease in the titers of IgA-, IgM-, and IgG-AMA were found only confined to patients with biochemical response to UDCA treatment ($P = 0.017$, $P = 0.047$, and $P = 0.005$, respectively). Notably, significant change for IgG-AMA was advanced to week 12 ($P = 0.005$). More intriguingly, similar pattern were also observed at week 24 in quantifying circulating plasmablasts ($P = 0.025$) and serum BAFF ($P = 0.013$). Additionally, immunohistochemistry staining of serial sections reflected an accumulation of CD38 + , IgA + , IgM + , and IgG + cells in portal tracts of PBC.

Conclusions: Decreased titers of serum IgG-AMA are associated with biochemical response to UDCA treatment, implicating a potential clinical utility of this classic hallmark in evaluating the therapeutic effect of UDCA for PBC patients.

O-084

Asymptomatic and symptomatic states are independent risk factors predicting PBC long-term outcome

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Background/aims: Primary biliary cirrhosis (PBC) is a liver-specific autoimmune disease. Patients are now being diagnosed at an asymptomatic stage due to increased awareness and earlier detection of this disease. We aimed to clarify the prognosis of PBC that was asymptomatic at diagnosis (ASym), as well as symptomatic PBC (Sym).

Methods: A total of 342 patients (292 women and 50 men, median age: 58, median follow-up period: 10 years) who were diagnosed between 1981 and 2013 were enrolled. We compared clinical findings with prognosis between the ASym and Sym groups.

Results: Of the 342 patients, 296 and 46 were ASym or Sym at diagnosis. Liver biopsy demonstrated Scheuer's stage I, II, III, or IV in 152, 50, 27, and 20 subjects. A total of 327 patients were treated with UDCA. Twelve patients required liver transplantation and 25 patients died. The ASym group was diagnosed at an early stage (85.8 %) more frequently than the Sym group (39.1 %). Plt, PT time, and Alb were significantly higher and AST, GGTP, and T-Bil and IgG were significantly lower in the ASym group ($p < 0.05$ for each factor). Survival at 10 and 20 years for the ASym and Sym groups were 97.3 % vs. 71.1 % and 88.7 % vs. 55.2 % ($p < 0.001$). Using a Cox proportional hazards model, ASym (Hazard ratio: 2.831; $p = 0.005$), early stage, albumin, and GGTP remained significant predictors of transplant or death.

Conclusions: ASym patients have a better prognosis than Sym. A regular screening program to identify patients with PBC at an early stage may be warranted.

O-085

Liver fibrosis progression only predicts survival and ursodeoxycholic acid response in PBC patients

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Background/aim: Response to ursodeoxycholic acid (UDCA) has shown to be a predictable factor for clinical outcomes of patients with primary biliary cirrhosis (PBC). We aimed to identify decisive factors of UDCA responders, and prognostic factors for long-term survival in PBC.

Methods: The 106 patients were divided into 2 groups, 53 responders and 53 non-responders, on the basis of median rate (69 %) of reduction in gamma-glutamyl transpeptidase (γ GTP) level 1 year after the commencement of UDCA. Univariate and multivariate analyses were performed to determine the difference between UDCA responders and UDCA non-responders in PBC. We also assessed correlation of histologic parameters with patient survival and response to UDCA. For evaluating the histological progression of PBC, we used two staging systems proposed by Scheuer and Nakanuma. Nakanuma scoring system consisted of bile duct loss (BDL) score and fibrosis score, which were independently scored from 0 to 3 on the basis of the magnitude.

Results: Logistic regression analysis revealed that Scheuer histological staging was the only factor distinguishing UDCA responders from UDCA non-responders. The Kaplan-Meier method showed that a poor prognosis could be predicted by Scheuer stage 3 and Nakanuma-fibrosis score 2 which were referred to as significant liver fibrosis. The proportion of UDCA responders to UDCA non-responders were significantly higher in PBC patients with Nakanuma stage 1–3 and Nakanuma-fibrosis score 0–2 than in those with Nakanuma stage 4 and Nakanuma-fibrosis score 3, respectively.

Conclusions: Long term prognosis and biochemical response to UDCA could be predicted by baseline liver fibrosis stage in PBC.

O-086

Assessment of quality of life of Japanese patients with primary biliary cirrhosis using PBC-40

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Background: Although it has been believed that majority of Japanese patients with primary biliary cirrhosis (PBC) are asymptomatic, patients could suffer of characteristic symptoms, such as fatigue and pruritus, known to reduce their individual quality of life (QOL). We conducted questionnaire-based survey of QOL in Japanese PBC patients.

Method: We used "PBC-40" questionnaire in Japanese version. The PBC-40 is a disease-specific QOL measure for self completion use in PBC. The PBC-40 has six domains: Fatigue, Cognitive, Social, Emotional, Itch and (other) Symptoms, and items are rated on an ordinal scale ranging from 1 to 5 (with high scores denoting the greater symptom impact and the worse QOL). The total score is obtained by averaging the 40 items. We asked patients to fill this questionnaire and send them back by mail.

Result: The questionnaire was retrieved from 217 Japanese patients with PBC (Male/female ratio: 19/198, age: 65.2 ± 9.5 years old). The average score was highest in Emotional domain (3.23), followed by Fatigue (1.26), Symptoms (1.01), Cognitive (0.91), Itch (0.90), and Social (0.49). When threshold determining impairment of QOL in each domain was set as 1.67 (one-third), the number of patients over this threshold was 35 in Symptom (17 %), 59 in Itch (29 %), 71 in Fatigue (34 %) and 117 in Emotional (57 %).

Conclusion: Overall, the QOL was impaired in Japanese PBC patients, especially in Emotional domain. With rigorous judgement of symptoms using PBC-40, symptoms related to PBC appear to be present in considerable part of Japanese PBC patients, more than previously reported.

O-087

Present status of primary sclerosing cholangitis in Japan: a nationwide survey

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Background: Primary sclerosing cholangitis (PSC) is a chronic cholestatic liver disease characterized by diffuse inflammation and fibrosis of bile ducts. In 2015, we conducted a nation-wide survey to elucidate present status of PSC in Japan.

Method: This survey is a questionnaires-based, multi-center retrospective study. Questionnaires were sent to the 211 major GI tertiary centers in Japan. The diagnosis of PSC basically was made according to the diagnostic criteria, mainly depending on cholangiographic studies, biochemical findings, and exclusion of other possible causes.

Result: We enrolled 416 patients with PSC in the current study; 219 patients who were newly registered and 197 patients already registered in the previous survey. Male/female ratio was 3:2, and the age distribution at diagnosis demonstrated two peaks, the 20s and the 70s. Almost half of patients were diagnosed asymptomatic, and serum value of alkaline phosphatase (ALP) was less than 2 times upper normal limit in 49 %. Cholangiography at diagnosis revealed that both intra/extra bile ducts were damaged in 56 %, and intrahepatic only in 36 %. Serum IgG4 level was elevated IgG4 in 12 %. As for complications, inflammatory bowel diseases were detected in 37 % overall, but 57 % in young patients (less than 45 yo). Five-year liver transplantation-free survival rate was 72.9 %.

Conclusion: We confirmed that (1) two peaks in age distribution at diagnosis, and (2) low complication rate of IBD as special features in Japanese PSC. Outcomes are still unfavorable and new drugs are strongly awaited.

O-088

Hepatitis B virus remains the leading cause of cirrhosis of liver in Bangladesh

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Aim: Our study presented at Kyoto APASL 2007 showed that HBV is the leading cause of cirrhosis in Bangladesh. Aim of present study was to see if there is any change in the etiology of cirrhosis in this country 8 years from Kyoto.

Methods: This is a retrospective study. Patients attending Hepatology Green Unit, Bangabandhu Sheikh Mujib Medical University, Dhaka in 2014 with cirrhosis were included.

Results: Total 752 patients were included. Of them 50.4 % [379] cases were due to HBV, 21.5 % [162] due to NASH, 15 % [113] due to HCV, 6.8 % [51] due to Wilson's disease, 3.6 % [27] due to alcoholic hepatitis and 2.7 % [20] due to autoimmune hepatitis. The figures remain same for both males and females. Although HBV remains the leading cause of cirrhosis in Bangladesh (61.2 % in 2007), NASH (3.6 % in 2007) has become more important HCV (2.2 % in 2007) as the etiology of cirrhosis in Bangladesh. We are also seeing more alcohol related cirrhosis in this country (1.5 % in 2007).

Conclusion: HBV is the commonest cause of cirrhosis in Bangladesh. Our government has integrated HBV vaccine into the existing Expanded Programme of Immunization. However we have to go a long way before we can sustain HBV in Bangladesh.

O-089

Necessity of anti-HBc and ALT screening for blood donors in Bangladesh

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Aim: Blood is checked for hepatitis B surface antigen (HBsAg) before transfusion at Bangladesh and most other developing countries of the world, because evidences have not provided from these countries to prove that HBsAg-negative blood may also harbor hepatitis B virus (HBV) DNA. In this study, HBV-infectivity was checked in HBsAg-negative blood.

Methods: Three hundred ninety eight HBsAg-negative apparent healthy blood donors were enrolled in this study. Antibody to hepatitis B core antigen (anti-HBc), hepatitis e antigen (HBeAg) and antibody to HBeAg (anti-HBe) were measured by ELISA method. Alanine aminotransferase (ALT) were also evaluated. HBV DNA was measured by polymerase chain reaction.

Results: Out of 398 HBsAg-negative blood donors, anti-HBc was detected in 82 apparently-health blood donors (20.6 %). Out of 82 HBsAg-negative, anti-HBc-positive sera samples, HBV DNA were detected in the sera of 7 blood donors (8.5 %). These 7 blood donor were negative for HBeAg and three subjects were expressing anti-HBe. The levels of ALT were more than 30 IU/L in 6 of 7 HBV DNA-positive subjects and it was above upper limit of normal (>42 IU/ml) in one blood donor.

Conclusion: The present study indicates that only checking of HBsAg is not enough to exclude HBV from blood donor. Although advanced countries have adopted nucleic acid testing (NAT) for preventing HBV transmission, developing countries may adopt anti-HBc testing and ALT estimation before proper atmospheres are attained for introducing NAT for blood transfusion.

O-090

Chronic hepatitis B in Australia: high prevalence of variants associated with disease progression

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Background: Hepatitis B virus (HBV) genotype and HBV variants including basal core promoter (BCP) and precore (PC) variants may influence the natural history of patients with CHB. However, HBV sequencing is not part of routine practice and there is little local data describing prevalence or clinical significance.

Aim: To determine the association between HBV genotype, common HBV variants, clinical features and virological profile in a large cohort of Australian patients with CHB.

Methods: A retrospective cross-sectional analysis of treatment-naive patients for HBV genotype, A1762T/G1764A BCP variant and G1896A PC variant was performed. HBV genotype / BCP/PC variant were correlated with age, gender, ethnicity, hepatitis B e antigen status (HBeAg), HBV replication status (ALT and HBV DNA), and liver fibrosis stage on biopsy. Advanced liver fibrosis was defined as METAVIR F3-4.

Results: 279 treatment naive patients were identified. They were predominantly male, Asian and HBeAg-negative with an average age of 41 years. There were significant differences between HbeAg positive and HBeAg negative cohorts (Table 1 attached). 53 % were in the immune escape phase of disease (Figure 1). Genotype B was most common (Fig 2) and genotype was strongly associated with ethnicity (Fig 3). There was a high prevalence of BCP or PC mutations (73 % see Fig 4). These variants were independently associated with age, male gender and advanced liver fibrosis (Table 2). **Conclusions:** The Australian CHB epidemic comprises 4 common genotypes, reflecting the multi-ethnic population. BCP / PC variants are common, and are independently associated with higher rates of advanced fibrosis.

Figure 1: Phases of disease

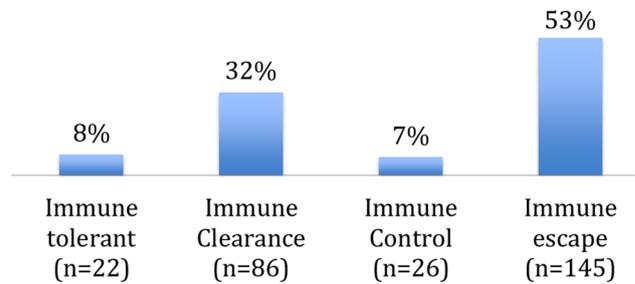


Figure 2: (above) Prevalence of genotypes in the cohort: Genotype B and C most common

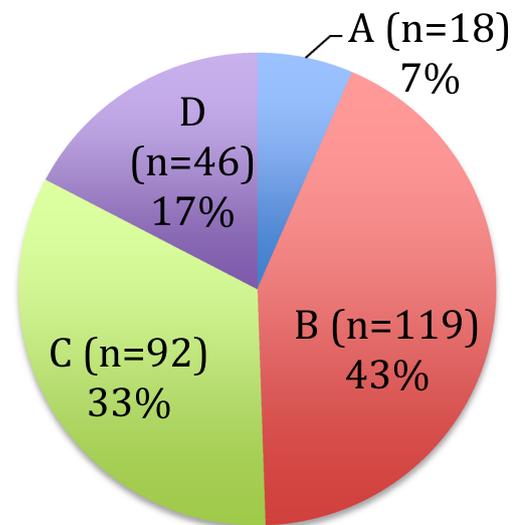


Table 1: Patient characteristics

	Overall	HBeAg +ve	HBeAg -ve	P value
Number	279	108 (40%)	171 (60%)	
Age, yrs (median, IQR)	41 (31-50)	34 (27-42)	46 (35-54)	<0.01
Male (n, %)	184 (66%)	61 (58%)	108 (68%)	
Ethnicity (n, %)				
Asian				
Caucasian	217 (78%)			
*Mediterranean	37 (13%)			
African	19 (7%)			
	10 (4%)			
		69 (85%)		
	37 (20%)	12 (15%)		

Figure 3 (above): strong correlation between genotype and ethnicity

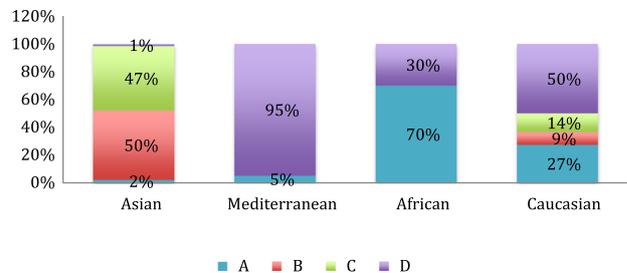
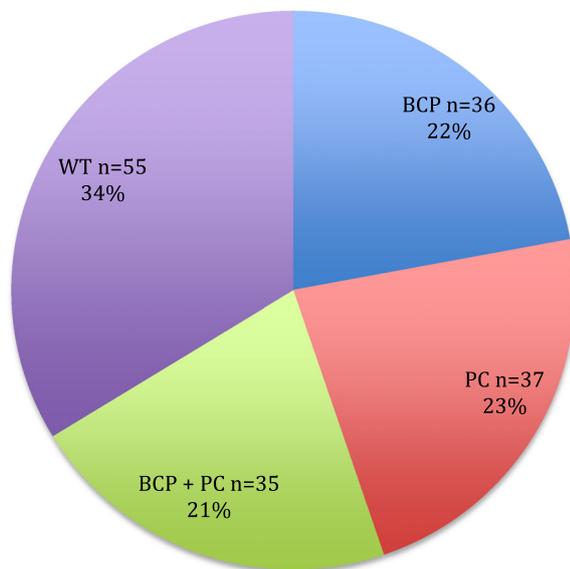


Fig 4: strong prevalence of mutations**Table 2: BCP/ PC mutations are independently associated with advanced fibrosis**

Univariable analysis				Multivariable analysis			
Effect	OR	95% Confidence Limits	P-value	Effect	OR	95% Wald Confidence Limits	P-value
Age > 40yrs	1.040	1.006 - 1.076	0.0211	Age > 40yrs	1.226	0.378 - 3.977	0.7438
Male	4.792	1.062 - 21.612	0.0415	Male	3.518	0.734 - 16.851	0.1155
HBeAg-pos vs. neg	0.620	0.246 - 1.568	0.3130	-	-	-	-
Genotype AD vs. BC	2.278	0.879 - 5.903	0.0901	Genotype AD vs. BC	1.721	0.607 - 4.878	0.3075
PC/BCP vs. WT	6.273	1.32 - 28.137	0.0165	PC/BCP vs. WT	5.178	1.065 - 25.181	0.0416

O-091

Hepatitis B virus infection decreases to a minimal extent after 30-years' universal vaccination**Yen-Hsuan Ni^{1,2}, Mei-Hwei Chang^{1,2}, Chi-Feng Jan³, Jia-Feng Wu¹, Hong-Yuan Hsu¹, Huey-Ling Chen¹, Ding-Shinn Chen²**¹Department of Pediatrics, National Taiwan University, Taipei, Taiwan; ²Hepatitis Research Center, National Taiwan University Hospital, Taipei, Taiwan; ³Department of Family Medicine, National Taiwan University, Taipei, Taiwan**Background/aim:** To monitor the impact of the Taiwan's world-leading hepatitis B virus (HBV) control program, we have conducted a series of seroepidemiologic surveys in Taipei metropolitan area. These surveys were performed every 5 years since 1984 when we launched the world's first universal HBV infant vaccination program.**Methods:** This was a descriptive analysis of HBV sero-markers in 4605 Taipei's residents aged from 0–50 years, approximately 100 subjects at each age level. They were recruited from the poster advertisement, newspaper propaganda, school screening, and well-baby clinics. All subjects were tested for serum HBsAg, its antibody (anti-HBs) and hepatitis B core antibody (anti-HBc). For those whose anti-HBc was positive, HBV genotyping and viral load were checked to detect occult HBV infection.**Results:** In the group <30 years of age (n = 3299), HBsAg prevalence was 0.5 %, and anti-HBs seropositive rate was 47.4 %. In contrast, HBsAg positive rate was 6.7 %, and anti-HBs seropositive rate was 69.4 % in the 30–50 age-group (n = 1306). Birth cohort analysis demonstrated no increase of HBsAg seropositivity for the vaccinees at the years of 30. For the HBsAg carriers below 30-year-old, 77 % were born to HBsAg carrier mothers. There was only one case of occult HBV infection in each group. Genotype C seemed to be more prevalent in the post-vaccination carrier population.**Conclusion:** Universal vaccination decreased the HBV carrier rate and infection rate. Mother-to-infant transmission accounted the majority of new HBsAg carriers in the post-vaccination era. Once overcoming this obstacle, we expect HBV-related diseases will be minimized in the decades to come.

O-092

The Hepatitis B Virus (HBV) HBx protein activates RSK2 to simultaneously regulate HBV replication**LiBo Yan^{1,2}, YouJia Yu³, YunHong Nong^{1,2}, Hong Tang^{1,2}**¹Center of Infectious Diseases, West China Hospital, Sichuan University, Chengdu, China; ²Division of Infectious Diseases, State Key Laboratory of Biotherapy, Sichuan University, Chengdu, China; ³Department of Forensic Pathology, Medical School of Basic and Forensic Sciences, Sichuan University, Chengdu, China**Aim:** Hepatitis B virus (HBV) X protein (HBx) play an important role in augmenting HBV transcription and replication via its transcriptional transactivation function which dependent on pleiotropic protein and protein interactions. This study was aim to investigate the cellular proteins of HBx-interactome in HBx regulating HBV replication.**Methods and results:** We have identified 71 HBx-related proteins through iTRAQ quantitative proteomics analysis about stable HepG2 cell lines with HBx different transactivation function domain pcDNA3.1-HBx, pcDNA3.1-HBx-Cm6, pcDNA3.1-HBx-Cm16. One of the identified protein is p90 ribosomal S6 kinase 2 (RSK2), which play an important role in MAPK Signaling Pathways. Up-regulation of RSK2 by HBV infection was confirmed by immunohistochemical in human liver tissue. HBx was found to increase RSK2 expression in either transient ectopic expression of HBx or a stably HBx expression cell line. Significantly, loss-of function studies showed that levels of HBV replication intermediates, HBsAg, HBeAg was decreased after silencing RSK2 in both HepG2.2.15 and HepG2 transfected with wild-type HBV construct. HBx has augmentation effects on HBV replication and expression as a HBx minus HBVmutant genome led to decreased levels of HBV replication intermediates, HBsAg, HBeAg and that these decreases can be restored to levels similarly with wild-type HBV by transient ectopic expression of HBx. After silencing RSK2 expression, the levels of HBV replication intermediates, HBsAg, HBeAg synthesized from HBx minus HBVmutant genome were not restored to levels that observed with wild-type HBV by transient ectopic expression of HBx.**Conclusions:** These results suggest that HBx activates RSK2 to simultaneously regulate HBV replication.

O-093

EFTUD2 restricts hepatitis B virus gene expression and replication through RIG-I mediated pathway

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Background and aims: Recently, RIG-I has been identified dually functions as a sensor in activating innate signaling to counteract viral polymerase in HBV infection (Sato S, et al. *Immunity*, 2015). We have reported that EFTUD2 exercised antiviral ability via RIG-I signaling pathway in HCV JFH1 culture system (Zhu C, et al. *J Virol*, 2015), but its role in HBV infection remains unclear. In this study, we evaluate whether EFTUD2 can restrict HBV replication through RIG-I mediated pathway in vitro.

Methods: We performed siRNA knockdown of RIG-I in HepG2.2.15 cells, and Huh7 cells transiently transfected with pHBV1.3. Selected gene expression were monitored by quantitative PCR and Western-blot. Levels of viral antigen expression and HBV DNA were measured by enzyme-linked immunosorbent assay and quantitative PCR, respectively.

Results: EFTUD2 overexpression inhibited HBV replication from $6,511,626 \pm 1,108,827$ to $3,030,405 \pm 403,128$ ($P = 0.0069$) in HepG2.2.15 cells, and meanwhile reduced HBsAg and HBeAg production by $58.9 \pm 9.1\%$ (13.49 ± 1.27 vs 5.54 ± 1.25 ; $P = 0.0015$) and $39.7 \pm 2.0\%$ ($22.862.0\% \pm 1.83$ vs 13.79 ± 1.34 ; $P = 0.0022$) in culture supernatants, respectively. We further confirmed EFTUD2's anti-HBV ability in another HBV replication system, namely Huh7 cells transfected with pHBV1.3. Moreover, EFTUD2 overexpression resulted in increased expression of RIG-I in both HepG2.2.15 and Huh7 cells transfected pHBV1.3, indicating RIG-I mediate EFTUD2's antiviral effect, which could be reversed by specific siRNA against RIG-I.

Conclusions: Our data demonstrate that EFTUD2 restricts HBV gene expression and replication through RIG-I mediated signaling pathways, and may provide a potential antiviral target for anti-HBV therapy in the future.

O-094

HBV-inducing miRNAs could affect the biological process and the expression of HBs-antigen

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Background: Previously, we reported that disease-specific miRNAs profiles in the peripheral blood by using deep sequencing (PLoS One

2013). Moreover, we reported that HBV-expression could induce stress-related proteins and chemokines from hepatocytes and modify the functions of immune cells (JID2010, JVI2015).

Aim: The aim of this study is to identify the HBV-specific expression of miRNAs in hepatocytes and exosomes and determine the biological significance of these miRNAs by comprehensive analysis.

Materials and methods: Study design: Permission for the study was obtained from the ethics committee at our institute. In vitro analysis: Huh7 and HepG2 cells were used for the transfection experiment of HBV expression. The exosomes were collected at 72 h after the transfection of HBV expression plasmids or vector plasmid. Illumina deep sequencing for the initial screening to determine the read numbers of miRNA expression in hepatocytes and culture supernatant was carried out. Ex vivo analysis: Total RNAs in the serum from chronic hepatitis B patients and healthy subjects were analyzed by deep sequencing.

Results: The network analysis indicated that regulation of the RNA metabolic process, the regulation of transcription etc. were significantly affected by the HBV expression in Huh7 cells and HepG2 cells. Moreover, the expression of miR125b-5p in HBV replicating Huh7, HepG2 cells and serum from CH-B was significantly higher than that in controls. The miR125b-5p could target the HBsAg and reduce the expression of HBsAg.

Conclusion: The HBV expression in hepatocytes can affect the expression of various kinds of miRNAs and modify the biological process of hepatocytes.

O-095

Restoration of CD56bright NK cells correlates with antiviral treatment efficacy in CHB patients

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Aim: To investigate the dynamic changes of NK cells post antiviral treatment and its potential influence on treatment efficacy.

Methods: This study involved 52 HBeAg—positive CHB patients who received telbivudine (Ldt) for 52 weeks. Blood samples were collected at baseline and week 12, 24, 36 and 48 of treatment, and tested for immunological markers.

Results: Compared with baseline, the percentages and absolute number of peripheral CD3-CD56 + NK cells were significantly higher from week 36 to week 48, especially CD3-CD56bright NK cells. The expression (percentage and MFI) of activating receptors NKG2D and NKp46 was enhanced, while inhibitory receptor NKG2A decreased. NKG2D expression was significantly enhanced on peripheral NK cells in patients with HBeAg seroconversion, particularly in CD3-CD56bright NK cell. The serum interleukin 15 (IL-15) level significantly elevated during Ldt treatment, especially in patients with HBeAg seroconversion. Co-culture of Ldt with purified peripheral NK cells from treatment naive HBeAg positive CHB patients showed significantly enhanced expression of NKG2D and IL-15.

Conclusion: Antiviral treatment by Ldt in CHB patients may play as an “adjuvant” agent capable of inducing or restoring NKG2D and IL-15 on circulating NK cell of HBeAg seroconversion patients.

O-096

HBV infection, persistence, immunity, and HLA and NTCP SNPs: an analysis of Taiwan Biobank data

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Background: We investigated associations of 4 candidate SNPs (rs2856718 and rs7453920 of HLA-DQ and rs3077 and rs9277535 of HLA-DP) and rs2296651 (S267F) of NTCP with HBV persistence (HBsAg serostatus), infection (anti-HBc), and immunity (anti-HBs) in Taiwanese population.

Methods: 6943 participants from the Taiwan Biobank were enrolled in this study, which is a community-based cancer-free cohort aged 30–70 years with GWAS data, derived using an Affymetrix customized Axiom array. Multivariate logistic regressions were performed with 95 % confidence intervals.

Results: A total of 868 (12.5 %) and 4108 (68.4 %) participants were seropositive for HBsAg and anti-HBc, respectively. Anti-HBs was seropositive for 964 (52.1 %) among anti-HBc-seronegatives. After adjustment for other SNPs, HBsAg-seropositivity significantly associated with HLA-DQ rs7453920 (OR = 0.58 [0.47–0.73] and 0.28 [0.09–0.92] for the GA and AA genotype) and HLA-DP rs9277535 (OR = 0.75 [0.62–0.90] and 0.44 [0.31–0.63] for the GA and AA genotype); anti-HBc-seropositivity with HLA-DP rs9277535 (OR = 0.78 [0.68–0.90] and 0.67 [0.54–0.83] for the GA and AA genotype) and NTCP rs2296651 (OR = 0.06 [0.03–0.13] for the AA genotype). In anti-HBc-seronegatives, anti-HBs-seropositivity significantly associated with HLA-DQ rs7453920 (OR = 0.68 [0.54–0.86] for the GA genotype), NTCP rs2296651 (OR = 0.48 [0.25–0.91] for the AA genotype), and HLA-DP rs9277535 (OR = 1.26 [1.02–1.54] and 1.51 [1.12–2.02] for the GA and AA genotype).

Conclusion: We confirm that candidate SNPs in HLA-DQ and HLA-DP are associated with HBV persistence. Both loci are associated with anti-HBs seropositivity. The HBV receptor homozygous variant (S267F) is strongly associated with reduced capability of HBV infection, while associated with decreased anti-HBs generation.

O-097

Hepatitis B virus core-related antigen as a surrogate marker for covalently closed circular DNA

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Background: Hepatitis B virus (HBV) covalently closed circular DNA (cccDNA) is a key to viral persistence in chronic hepatitis B patients. We aimed to explore whether serum hepatitis B core-related antigen (HBcrAg) could be a surrogate marker for intrahepatic cccDNA in a large cohort of patients with liver biopsies.

Methods: 305 liver biopsies and the corresponding sera collected from 138 nucleoside/tide analogues-treated patients were analyzed.

124 patients had paired liver biopsies at baseline and 1 year post-treatment, and 43 patients had a third biopsy after >5 years of treatment. Serum HBcrAg, HBV DNA and hepatitis B surface antigen (HBsAg), and intrahepatic cccDNA were measured.

Results: HBcrAg strongly correlated with cccDNA ($r = 0.695$), serum HBV DNA ($r = 0.691$), and HBsAg ($r = 0.449$; all $p < 0.00001$). Serum HBV DNA were undetectable (<20 IU/mL) in 120 samples. In these 120 samples, HBcrAg also correlated positively with cccDNA ($r = 0.388$, $p = 0.00001$). At baseline, all 124 patients had HBcrAg >1 kU/mL (median level: 3.6 log kU/mL). At year 1 and >5 years post-treatment, the median HBcrAg levels were 2.3 and 0.77 log kU/mL, with a median logarithmic reduction of 1.4 and 2.7 log kU/mL (compared with baseline), respectively. 21 patients had undetectable cccDNA (<0.005 copies/cell) after >5 years post-treatment, in which 15 (71 %) had HBcrAg levels >1 kU/mL (range 1.2–537 kU/mL).

Conclusions: Serum HBcrAg might be a potentially reliable surrogate marker for intrahepatic cccDNA content even when serum HBV DNA became undetectable. Viral proteins were still synthesized in the majority of patients with undetectable cccDNA after long-term treatment.

O-098

5 year efficacy and safety of tenofovir DF in lamivudine-resistant chronic hepatitis B patients

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Background: In CHB patients with lamivudine-resistance (LAM-R), TDF showed comparable efficacy to FTC/TDF and no resistance at 2 years.

Methods: CHB patients with HBV DNA >3 log₁₀ IU/mL and LAM-R (INNO-LiPA) were randomized to TDF or FTC/TDF for 5 years.

Results: Of 280 patients randomized, 239 (85 %) completed 5 years. Baseline (BL) characteristics were: mean age 47 years, 75 % males, 34 % Asians; 53 % HBeAg-, HBV genotypes (A-D) 22, 13, 19, and 43 %; mean (SD) HBV DNA 5.7(1.9) log₁₀ IU/mL; 42 % with ALT < ULN. At Year 5, 83 and 83 %, 65 and 71 %, 12 and 10 %, 1.4 and 3.6 %, had HBV DNA <69 IU/mL, ALT normalization, anti-HBe+ and HBsAg loss for TDF and FTC/TDF, respectively ($p = ns$). Nine patients (4-TDF, 5-FTC/TDF) discontinued for an AE. Overall, 3 (1 %) patients developed HCC. Renal endpoints (both groups) were: CrCL < 50 mL/min $n = 19$ (7 %), increase from BL in serum creatinine of >0.3 or >0.5 mg/dL, $n = 21$ (8 %) and $n = 2$ (<1 %), respectively; serum phosphorus <2 mg/dL, $n = 3$ (1 %) patients. Mean declines from BL in BMD (g/cm²) (both groups) for hip and spine were 3, and 1 % at Year 5. Seven patients experienced fracture (6 trauma-related). No TDF resistance was detected by population sequencing.

Conclusions: A high rate of viral suppression was maintained with TDF for 5 years with no resistance detected; FTC/TDF offered no

advantages in this setting. Renal events occurred in up to 8 % of patients, and average BMD losses of up to 3 % were observed.

O-099

No detectable TDF resistance when given alone or with FTC in CHB patients with LAM-R: 5 year results

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Aim: Compare amino acid changes within HBV polymerase/reverse transcriptase (pol/RT) after up to 5 years (240 weeks) of treatment with TDF or FTC/TDF.

Methods: Enrolled were patients receiving LAM and harbored LAM resistance mutations (LAM-R) (rtM204 V/I ± rtL180 M) who were randomized 1:1 to TDF or FTC/TDF for up to 240 weeks. Virologic breakthrough (VB) was defined as confirmed HBV DNA >1 log₁₀ increase from nadir or HBV DNA ≥69 IU/mL after <69 IU/mL. Population sequencing of HBV pol/RT was attempted for all patients at baseline and annually or at discontinuation if viremic (HBV DNA ≥69 IU/mL).

Results: 280 patients entered the study (TDF = 141, FTC/TDF = 139). Four TDF patients (2.8 %) were viremic at year 5/ last visit (median [range] HBV DNA 2.31 log₁₀ IU/mL [1.88–6.46]), with 1 experiencing VB (associated with study medication nonadherence). The VB patient had unique polymorphic site changes, while the remaining patients had no change (n = 1) or were unable to be genotyped (n = 2). Seven FTC/TDF patients (5.0 %) were viremic at year 5/ last visit (median [range] HBV DNA 5.50 log₁₀ IU/mL [1.91–9.19]), with none experiencing VB. Three FTC/TDF patients had conserved site changes (rtA87A/G [n = 1], rtM/I204I [n = 1], rtI233 V [n = 1]), 1 had unique polymorphic site changes, and 3 had no change. Phenotypic analysis demonstrated no TDF resistance. Neither baseline ETV-R (N = 25) nor prior ADV (N = 61) or ETV (N = 13) treatment had an impact on viral kinetics.

Conclusions: TDF monotherapy was as effective as FTC/TDF combination therapy in CHB patients harboring LAM-R; no TDF resistance was detected through 5 years of treatment.

O-100

HBs antigen reduction by peg-interferon sequential therapy after long term NUC

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Background and aims: The loss of HBs antigen (HBsAg) is rarely achieved by nucleot(s)ide analogues (NUC) therapy. The aim of the present study is to elucidate the effect of peg-interferon on HBsAg reduction when given sequentially after long term NUC therapy.

Methods: 48 weeks of peg-interferon alpha-2a were sequentially given to 54 patients after long-term NUC therapy. Preceding the

average duration of preceding NUC therapy was 4.1 years. Serial changes in the titer of HBsAg were measured by quantitative assay. **Results:** By sequential therapy, HBeAg became negative at the end of therapy in 50 %. The titer of HBsAg was 850 (1.9–36,500) IU/mL at baseline which declined to 166 (<0.05–22500) IU/mL at the end of therapy. The incidence of HBsAg reduction >1.0 Log IU/mL was 34 %, HBsAg reduction >2.0 Log IU/mL was 16 %, and HBsAg loss was 6 %. Within the same individual, average HBsAg reduction during sequential peg-interferon was significantly higher compared to those during preceding NUC therapy (p = 0.015). Among patients with HBsAg <1000 IU/mL at the start of therapy, average HBsAg reduction during therapy was 0.96 Log IU/mL.

Conclusions: Peg-interferon given sequentially after long term NUC therapy could be a strategy alternative to life-long NUC maintenance therapy since the speed of HBsAg reduction accelerated during sequential peg-interferon therapy compared to preceding NUC therapy.

O-101

Switching to PegIFN α -2a in NUC treated CHB patients (NEW SWITCH study): comparison 48 and 96 weeks

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Objectives: Switching to PegIFN α from NUC has been shown to be safety and promising with higher HBsAg loss rate compared to continuous NUC therapy. This study aims to evaluate whether to prolong the treatment duration of PegIFN α -2a from 48 to 96 weeks could improve HBsAg loss rate.

Method: This was a Phase IV, open-label, randomized study conducted at 25 centers in China. HBeAg positive CHB patients who had received adefovir, lamivudine or entecavir for 1–3 years and achieved partial response (defined as HBV DNA <200 IU/ml and HBeAg loss) were randomised 1:1 to receive PegIFN α -2a for 48 (Arm A) or 96 (Arm B) weeks with NUC therapy in first 12 weeks and followed-up for 48 weeks after treatment. The primary endpoint was HBsAg loss at the end of treatment.

Results: Of 304 patients randomized, 303 patients received ≥ 1 study drug dose were analyzed (ITT). Patients of Arm B showed higher HBsAg loss (21.3 vs 16.3 %) and HBsAg seroconversion (16.0 vs 14.4 %) than those of Arm A at the end of treatment. The rates of ALT normalization (61.3 vs 51.3 %) and HBeAg loss and seroconversion (60.7 vs 55.6 %) in Arm B were also higher than Arm A. PegIFN α -2a was well tolerated. Only 3 patients (1.0 %) experienced ALT flare (increased to >5 ULN). 32 subjects (10.6 %) experienced virological breakthrough (HBV DNA increased >1 log IU/ml from nadir).

Conclusion: Prolonged treatment duration of switching to PegIFN α -2a may increase HBsAg loss rate in HBeAg positive CHB patients treated with long-term NUC.

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PegInterferon combination therapy versus no treatment in HBeAg-negative low viral load CHB patients

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Aims: Chronic hepatitis B (CHB) patients with a low viral load (LVL) are currently not eligible for antiviral treatment. Here we present a randomized trial comparing combination treatment of peginterferon alfa-2a (peg-IFN) and a nucleotide analogue versus no treatment for CHB patients with LVL.

Methods: 134 CHB patients (HBeAg-negative, HBV-DNA <20,000 IU/mL) were randomized 1:1:1 to receive peg-IFN + adefovir (arm I; n = 46), peg-IFN + tenofovir (arm II; n = 45) or no treatment (arm III; n = 43) for 48 weeks. Randomization was stratified by HBV genotype A (22 %), non-A (B 7 %, C 4 %, D 26 %, E/F/G 20 %), or indeterminable (21 %). Twelve patients discontinued the study before week 72.

Results: At end of follow-up (week 72), 4 patients receiving combination therapy had achieved HBsAg loss, compared to none of the untreated patients (4.4 vs 0 %, p = 0.31). HBsAg levels had declined significantly in all arms at week 72; -0.53 (p < 0.001), -0.59 (p < 0.001), and -0.15 (p < 0.001) mean log₁₀ IU/mL reduction for arms I, II, and III, respectively. HBsAg declined more in treatment arms I (p = 0.004) and II (p = 0.004) compared to the control arm

III. On-treatment ALT increase as well as week 12 HBsAg level were significant predictors of on-treatment HBsAg decline in multivariable models.

Conclusion: In CHB patients with LVL, 48 weeks of peg-IFN based combination treatment resulted in 4.4 % HBsAg loss, compared to 0 % in untreated controls. A significant decline in HBsAg at week 72 may indicate a further increase in HBsAg loss during extended follow-up.

O-103

Effective inhibition of cccDNA derived mRNA/viral antigens and tolerability with ARC-520

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Background: ARC-520, an RNA interference drug, targets cccDNA-derived mRNA in chronic hepatitis B patients (CHB); herein we report tolerability/activity in normal healthy volunteers (NHV) and CHB.

Methods: 54 NHV (36 ARC-520, 18 placebo) and 58 CHB (48 ARC-520, 10 placebo) were included. At entry, 32 of 38 HBeAg-neg and 14 of 20 HBeAg-pos CHB had taken entecavir for mean 5 years (range 2–8) and continued throughout the study. NHV and CHB received 0.01 to 4 mg/kg IV ARC-520 or placebo; CHB had viral parameters measured.

Results: ARC-520 therapy was well tolerated—67 % NHV (placebo or ARC-520) and 23 % CHB reported a mild or moderate adverse event (AE) with no AE rated serious, severe, or causing withdrawal. A modest occurrence of abnormal laboratory tests was observed. Two NHV showed moderate hypersensitivity reactions (urticarial rash, flushing) during infusion. After addition of pretreatment oral antihistamine, no hypersensitivity reactions were seen. ARC-520 reduced viral antigens with qHBeAg best reduction of 1.7 log (mean max 1.2 log) following a single 4 mg/kg dose. In treatment-naïve CHB, best qHBsAg reductions of 1.9 log (mean max 1.3 log) in HBeAg-pos and 0.8 log (mean max 0.3 log) in HBeAg-neg were observed. Similar reductions in HB core related antigen were observed where measurable.

Conclusions: (1) ARC-520 effectively inhibited cccDNA-derived mRNA with protein reductions up to 1.9 logs (99 %) (2) ARC-520 was well tolerated and hypersensitivity symptoms were controlled by antihistamine pretreatment. (3) This is the first time direct antiviral effects on HBeAg and HBsAg have been demonstrated.

O-104

The risk factors for reactivation of Hepatitis B virus after hematopoietic stem cell transplantation

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Background and aim: The aim of this study was to determine the risk factors for HBV reactivation after allogenic hematopoietic stem cell transplantation (HSCT).

Methods: Among 519 allogenic HSCT patients between 2004 and 2014, 52 patients with resolved HBV infection (Hepatitis B s antigen negative, hepatitis B core antibody [HBcAb] and/or hepatitis B surface antibody positive) were recruited to follow up for HBV reactivation. To identify predictors of HBV reactivation after HSCT, the medical record were reviewed for covariates and outcomes of interest including: age, sex, primary disease, conditioning regimen, donor relatedness, human leukocyte antigen donor matching, stem cell source, GVHD prophylaxis regimen, development and maximal grade of acute GVHD, development of chronic GVHD (cGVHD), pretransplant HBcAb and HBsAb measurements, pre- and post-HSCT corticosteroid treatment, and cessation of posttransplant immunosuppressive drug.

Results: Fourteen of the 52 patients (26.9 %) had HBV reactivation after HSCT. The median interval between HSCT and HBV reactivation was 15 months (3–68). In univariate analyses, factors for HBV reactivation with significance were: titer of HBcAb ($P = 0.000$), cGVHD ($P = 0.043$), and pre-HSCT steroid therapy ($P = 0.012$). In multivariate analyses, HBcAb ≥ 8 S/CO (HR, 6.957; 95 % CI, 1.772–27.319; $P = 0.005$) and pre-HSCT steroid therapy (HR, 3.822; 95 % CI, 1.139–12.832; $P = 0.030$) were independent factors for HBV reactivation. Cumulative rates of HBV reactivation at 1, 3, 5 years after HSCT were 10.8, 30.6 and 43.9 %, respectively.

Conclusion: HBcAb ≥ 8 S/CO and pre-HSCT steroid therapy are risk factors for HBV reactivation after allogenic HSCT.

O-105

Metabolic syndrome increases CV event but not hepatic event and death in chronic hepatitis B

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Background and aims: Metabolic syndrome (MES) increases the risk of cirrhosis on chronic hepatitis B (CHB) patients. Whether CHB patients with MES would be predominantly affected by hepatic or cardiovascular (CV) event remains unknown. We aimed at investigating the impact of MES on long-term outcomes of CHB patients.

Methods: This was a prospective cohort study of 1466 CHB patients, who had baseline assessment including detailed metabolic profiling and liver stiffness measurement (LSM) by transient elastography in year 2006–2008. Patients were prospectively followed up for any clinical events. The impact of LSM and MES on hepatic event, CV event or death was evaluated.

Results: The baseline mean age was 46 ± 12 years. The ALT level was 72 ± 139 IU/l, 74 % had negative HBeAg; HBV DNA level was 5.2 ± 2.0 log₁₀ IU/ml. LSM value was 8.4 ± 6.3 kPa. Patients with baseline LSM >8.0 kPa had higher cumulative probability of hepatic events than those with LSM <8.0 kPa at 8 years (12.3 vs. 3.1 %; $P < 0.001$). Patients with MES had higher cumulative probability of

CV events than those without (8.0 vs. 2.2 %; $P < 0.001$). High LSM did not impact on CV events; neither MES on hepatic events. LSM >8.0 kPa but not MES was an independent risk factor of death, with an adjusted hazard ratio 1.9 (95 % confidence interval [CI] 1.1–3.2) and 1.3 (95 % CI 0.8–2.4) respectively.

Conclusions: MES increases risk of CV event but not hepatic event or death in CHB patients, whereas high baseline LSM was a major independent risk factor of hepatic event and death.

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Prognosis of patients with chronic Hepatitis B and chronic kidney disease: a 6-year cohort study

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Background: Chronic kidney disease (CKD) is prevalent among patients with chronic hepatitis B (CHB). Our aim was to report prognosis of patients with CHB and CKD.

Methods: We analyzed claims data in the French National Hospital Database for the years 2008 to 2013, with all diseases classified using ICD-10 codes. A total of 47,586 adults in Metropolitan France who had CHB were recorded at January 2008. Of these, 4178 (8.8 %) patients were discharged with CKD. Adjusted hazard ratio (aHR) estimates for a composite outcome of liver transplant or death were calculated by multivariate Cox models after adjusting for competing kidney transplant.

Results: The majority of patients were male (69.0 %) male and had risk factors for CKD (85.2 %) including high blood pressure, diabetes, obesity, or acute kidney injury. Mean (SD) age was 60 (15) years at first CKD discharge. HIV infection was present in 15.0 % of patients. During the study period: 377 (9.0 %) developed hepatocellular carcinoma; 1171 (28.0 %) end-stage liver disease; 148 (3.5 %) hepatorenal syndrome; and 1205 (28.8 %) end-stage kidney disease. At 6-year follow-up, 1249 (29.9 %) patients had died and 139 (3.3 %) had received a liver transplant. Hepatocellular carcinoma (aHR = 2.4), end-stage liver disease (aHR = 2.2), hepatorenal syndrome (aHR = 2.0), and end-stage kidney disease (aHR = 1.9) were independent risk factors of liver transplant or death. The likelihood of receiving a liver transplant increased independently with hepatorenal syndrome ($p < 0.001$) but not with end-stage kidney disease ($p = 0.26$).

Conclusion: CKD has a dismal prognostic value in patients with CHB.

O-107

Co-treatment with pegylated interferon alfa 2a and entecavir for hepatitis D: a randomized trial

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Background: The aim of this study was to investigate the efficacy of PEG-IFNa-2a therapy with and without entecavir in patients with chronic hepatitis D.

Methods: This study included 40 HDV RNA-positive patients. Patients were randomized to receive either PEG-IFNa 2a 180 microgram weekly in combination with entecavir 0.5 mg daily (n = 21) or PEG-IFNa alone (n = 19). Patients who failed to show 2 log reduction in HDV RNA level at 24 weeks of treatment, or had detectable HDV RNA at 48 weeks of therapy were considered as treatment failure. Treatment was continued for 72 weeks in the rest of the patients. All the patients were followed up for 24 weeks post treatment. Intention to treat analysis was performed.

Results: The mean age of the patients was 26.7 ± 6.8 years, 31 were male. Two log reduction in HDV RNA levels at 24 weeks of therapy was achieved in 9(43 %) patients receiving combination therapy and 12(63 %) patients receiving PEG-IFNa alone (p = 0.199) who became eligible to continue the treatment. Decline of HBsAg levels was insignificant. At the end of treatment, HDV RNA was negative in 8 patients (38 %) receiving combination therapy and 10 patients (53 %) receiving PEG-IFNa-2a alone. Virological response persisted in 7(33 %) and 8(42 %) patients respectively at the end of follow up period while both virological and biochemical responses were seen in 5(24 %) and 7(37 %) patients.

Conclusions: Administration of PEG-IFNa-2a with or without entecavir, resulted in persistent HDV RNA clearance in 37 % of patients. Addition of entecavir did not improve the overall response.

O-108

High rates of surface antigen loss, low rates of seroreversion in Asian chronic HBV patients

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Background: Chronic hepatitis B virus (HBV) infection is a global disease with the majority from Asian-Pacific regions. It is unclear if HBV surface antigen (HBsAg) loss alone correlates well with undetectable virus, normalization of alanine aminotransferase (ALT) on treatment, future development of anti-HBs, nor is the risk of seroreversion or detectable virus after stopping therapy well defined.

Methods: We retrospectively evaluated 958 chronic HBV adults on oral antiviral therapy at a community gastroenterology clinic from 1997–2015. 99.6 % were Asian ethnicity. Rates of HBsAg loss, ALT normalization, achieving undetectable virus, and developing surface antibody (anti-HBs) were stratified by HBeAg status. Following HBsAg loss, rates of HBsAg seroreversion and/or re-emergence of detectable virus were analyzed.

Results: With a mean 86.6 months follow-up and mean treatment duration of 81.2 months, overall HBsAg loss was 4.91 % (n = 47), with similar rates between HBeAg positive (4.43 %) and negative patients (5.14 %), p = 0.63. Among HBsAg loss patients, 31.8 % developed anti-HBs, 97.9 % achieved undetectable virus, 66.0 % normalized ALT. 19 patients had both HBsAg and anti-HBs positivity. 2.1 % (n = 1) who achieved HBsAg loss had detectable virus, whereas 12.8 % (n = 6) of HBsAg loss patients seroreverted with positive

HBsAg within 1–17 months. All 6 patients who seroreverted had sustained undetectable virus, and 1/6 had positive anti-HBs prior to seroreversion. Anti-HBs status did not predict HBsAg seroreversion.

Conclusion: Among this Asian patient population, treatment of chronic HBV achieved 4.91 % HBsAg loss. While 12.8 % of patients experienced seroreversion, all maintained undetectable virus, demonstrating the sustainability of HBsAg loss in most patients and stable suppression of HBV virus.

O-109

Host and viral determinants for differential hepatitis B virus clearance between genotypes

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Background: Hepatitis B virus (HBV) is categorized into nine genotypes (A–H, J), and the clinical characteristics differ among genotypes. Very little is known about the immunological basis for such difference.

Methods: To examine whether differential HBV clearance rate between genotypes is associated with HBV-specific CD8 T cell responses in the liver, HBV genotype C and D were introduced into C57BL/6J mice by hydrodynamic injection of plasmids containing a replication competent 1.24-fold HBV-DNA. Serum HBs antigen (HBsAg) was monitored on days 1, 4 and 14 after hydrodynamic injections, and intrahepatic expressions of HBV-DNA, HBV-mRNA, and HBeAg were examined on days 4 and 14. HBV-specific CD8 T cell responses in the liver was examined on day 14 to correlate with the aforementioned viral parameters.

Results: Serum HBsAg as well as intrahepatic HBV-DNA and HBV-mRNA expressions persisted longer in the mice expressing genotype C than genotype D. The delayed clearance of genotype C was associated with the weaker intrahepatic CD8 T cells on day 14, and the lower intrahepatic HBeAg expression on day 4. Interestingly, when its cognate HBeAg expression was transcomplemented by a plasmid expressing only HBeAg, the clearance of genotype C was accelerated in association with robust induction of HBV-specific CD8 T cell responses.

Conclusions: The rate of HBV clearance could differ between genotypes. The differential clearance rate is associated with the HBeAg expression levels that determine the magnitude of HBV-specific CD8 T cell responses.

O-110

Application of highly sensitive CLEIA for HBsAg: an appropriate method to detect occult HBV viremia

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Aim: We established highly sensitive chemiluminescent enzyme immunoassay (CLEIA) for detection of hepatitis B surface antigen (HBsAg). This assay uses for quantitative HBsAg detection by combining monoclonal antibodies, each specific for a different epitope of the antigen, and engaging a developed conjugation technique. Here, we determine that highly sensitive HBsAg CLEIA (Lumipulse HBsAg-HQ) is a precise system to detect occult HBV infection.

Methods: The sensitivity of Lumipulse HBsAg-HQ (Fujirebio, Inc.) is nearly 6-fold higher than that of quantitative HBsAg detection system HISCL HBsAg (Sysmex). In this study, the performance of Lumipulse HBsAg-HQ was compared with that of HISCL HBsAg. This study protocol was approved by the appropriate institutional ethics review committees.

Results: In our hospital, HBsAg of 4733 serum samples were measured by HISCL HBsAg between July 1st 2013 and February 28th 2015 for pre-transfusion testing. Of the 4733 samples, 117 samples (2.5 %) were HBsAg seronegative but showed low concentration of HBsAg (10 to 20 mIU/mL). Of the 117 samples, 17 (14.5 %) were positive for anti-hepatitis B core antigen (anti-HBc), and 15 of the 17 samples were available for further assay. They were measured by Lumipulse HBsAg-HQ retrospectively and 3 (20 %) were detectable for HBsAg. Two samples were from chronic hepatitis B carriers with HBsAg sero-clearance and one was from a leukemia patient diagnosed as HBV reactivation. Their HBsAg concentrations by Lumipulse HBsAg-HQ were 8.0, 38.5 and 7.2 mIU/mL, respectively.

Conclusion: Lumipulse HBsAg-HQ is an accurate method to detect occult HBV viremia as well as HBV reactivation at early phase.

O-111

Detection Of HBsAg, HBeAg And HBV DNA load in saliva of HBV carriers

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Background/objectives: HBV can be transmitted by parenteral exposure to different human body fluids. This study is designed to test for HBsAg, HBeAg and HBV DNA load both in saliva and serum simultaneously to look for diagnostic value of saliva in HBV infection.

Methods: 60 HBV carrier patients were stratified according to their epidemiological profiles and risk factors. Blood and saliva samples of those cases were tested simultaneously for HBsAg, HBeAg, and HBV DNA load to determine sensitivity and specificity of saliva for detection of these serological and molecular markers.

Results: 11.67 % patients had H/O blood transfusion. 20 and 16.7 % had evidence of perinatal transmission and sexual transmission. Salivary detection of HBsAg have a sensitivity of 91.67 % and a PPV of 100 %. Salivary detection of HBeAg found to have a sensitivity of 78.57 % and specificity of 100 %. Majority of HBV carriers (33.33 %) had serum HBV DNA level between $(10 \times 3-10 \times 4$ copies/ml). Salivary detection of HBV DNA had a sensitivity of 39.58 % and specificity of 100 % but DNA level was very less compared to serum. Mean HBV DNA load in serum was log 3.59 copies/ml and in saliva was log 3.34 copies/ml.

Conclusions: Perinatal transmission, transfusion related transmission and sexual transmission are the major modes of transmission of HBV in our country. Testing of saliva for HBsAg has got a high sensitivity and specificity. Salivary detection rate of HBV DNA is not very high but there is a significant correlation between serum and salivary HBV DNA level.

O-112

Alteration of hepatitis B virus release after the disruption of clathrin-dependent endocytosis

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It has been reported that the multivesicular body (MVB) functions are required for the assembly/release of hepatitis B virus (HBV). Because endocytotic pathway is known to be required for the maintenance of MVB membrane, it is speculated that endocytosis is important for the assembly/release of HBV particles as well as the viral entry. In this study, we disrupted the pathway of clathrin-mediated endocytosis (CME) and observed changes of HBV release using HepG2.2.15 cells expressing HBV particles stably. Interestingly, siRNA-mediated depletion of clathrin heavy chain, adaptin-alpha, Rab5A, Rab5B, and Rab5C increased the release of HBV particles. Adaptin-alpha is known as a component of AP2, which is an adaptor protein of clathrin. Rab5 proteins are small GTPases that are acting as molecular switches of trafficking between plasma membrane and early endosomes. The density of HBV particles after Rab5B depletion was changed from 1.24 to 1.18 g/ml, but they retained infectivity to HepG2 cells expressing NTCP. Because it has been reported that the depletion of Rab5 decreased the number of MVBs, which is an authentic organelle for the HBV assembly, it was thought that the changes after depletion of CME-associated proteins might indicate the presence of alternative release pathway of HBV.

Conclusion: In this study, it was shown that the disruption of CME increased the release of HBV particles that had lower density. These data suggest that CME might be required for the authentic pathway of HBV assembly/release, and the disruption of CME might enhance an aberrant release of HBV.

O-113

PreS mutations are correlated with the progression of liver diseases and HBsAg/HBcrAg productions

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Background and aim: PreS region of HBV genome responsible for HBsAg production was reported to be often mutated in advanced liver disease. On the other hand, HBsAg and hepatitis B core-related

antigen (HBcrAg) is known to reflect HBV replication activity. We analyzed the interrelationship among HBsAg titer, HBcrAg titer, preS mutations and disease progression.

Methods: A total of 96 patients with 32 inactive carrier status (IC), 28 chronic hepatitis (CH) and 36 cirrhosis or hepatocellular carcinoma (LC/HCC) were analyzed. They were all nucleotide analogue naïve. Deep sequencing was performed targeting 522nt in the preS region, and proportion of preS1 and preS2 deletions was determined in each patients.

Results: Median HBsAg was respectively 688 (IC), 2075 (CH), and 1065 IU/ml (LC/HCC), and median HBcrAg was respectively 3.0, 4.9, and 5.85 logIU/ml. The proportion of patients with preS1 deletion was higher in CH and LC/HCC (IC 12 %, CH 36 %, LC/HCC 28 %: $p = 0.03$). The proportion of patients with preS2 deletion was higher in CH and LC/HCC (IC 19 %, CH 54 %, LC/HCC 61 %: $p < 0.01$). While the proportion of preS deletions in each patients was inversely correlated with HBsAg titer in LC/HCC patients (preS1 $p < 0.01$, preS2 $p = 0.04$), it was positively correlated with HBcrAg titer (preS1 $p = 0.03$, preS2 $p < 0.01$).

Conclusions: PreS mutations are associated with liver disease progression and with the decrease of serum HBsAg, and HBcrAg. Importantly, it was disclosed even patients with low HBsAg are still at risk for advanced liver disease, suggesting that strategies based on preS mutation should be established.

O-114

Sequential therapy with short-term entecavir followed by interferon in chronic hepatitis B patients

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Guidelines in Japan basically recommend interferon- α as the first-line especially for young patients with chronic hepatitis B, and priming of short-term nucleos(t)ide analog is considered when HBV DNA is 7.0 log copies/mL or higher. Forty-eight patients with chronic hepatitis B (38 men and 10 woman; 39 ± 7 years old) received entecavir for 36–52 weeks, followed by entecavir plus interferon- α for 4 weeks, and lastly by interferon- α alone. In 24 patients enrolled before 2012, nonpegylated interferon- α was given for 24 weeks in total (Non-PEG group), and in 24 patients enrolled thereafter, pegylated interferon- α was given for 48 weeks (PEG group). The proportion of HBeAg-positive patients was higher (100 vs 58 %, $P = 0.0006$) in the Non-PEG group than in the PEG group. In the Non-PEG group and in the PEG group, respectively, mean ALT level (IU/L) was 25 ± 11 and 22 ± 10 , HBeAg-positive rate (%) was 71 and 54, and mean HBV DNA load (log copies/mL) was 2.3 ± 0.4 and 2.3 ± 0.4 at the start of interferon- α ; ALT (IU/L) was 35 ± 23 and 56 ± 64 , HBeAg-positive (%) was 67 and 21, and HBV DNA (log copies/mL) was 3.4 ± 1.7 and 3.3 ± 2.4 at the end of interferon- α ; and ALT (IU/L) was 212 ± 241 and 109 ± 235 , HBeAg-positive (%) was 63 and 36, and HBV DNA (log copies/mL) was 6.4 ± 2.2 and 5.6 ± 2.6 at 24 weeks post-treatment. HBsAg seroclearance was achieved in one patient of the PEG group. Our results suggest that the use of pegylated interferon- α for 48 weeks is superior to the use of non-pegylated interferon- α for 24 weeks in sequential therapy following short-term entecavir priming.

O-115

CHB patients long term followed up for cirrhosis & HCC development under tenofovir treatment

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CHB infection is one of important global health problem causing liver cirrhosis, hepatocellular carcinoma, liver related mortality and morbidity. Tenofovir has proven its efficacy over viral suppression and reversal of liver cirrhosis. Weather treatment with nucleos(t)id analogs protect the patients from liver cirrhosis and hepatocellular carcinoma or not are controversial.

Aim of the study: To investigate cirrhosis and HCC development rate under the long term treatment with TDF.

Patients and method: This is 3 years retrospective, 2 years prospective multi-centric cohort study. 357 patient has been enrolled to study, 246 (68.9 %) of them are male, mean age 48.3 year were followed up for 64.5 months. At the baseline of study, 7 cirrhotic patients have been enrolled and none of them has progressed during study. The patients were checked for liver function tests, AFP, upper abdominal ultrasonography in every 6 months.

Results: HBV DNA suppression rate 88.1 %, ALT and AST normalization rate were 83, 91.6 % respectively. Seven patients have developed cirrhosis. HBeAg(+), hepatosteatois, male gender and obesity are common reasons for cirrhosis. Cirrhosis incidence was 0.36 person-years. Cirrhosis development rate is very low in comparison to historical cohorts. HCC did not occur (0 %) in any patient during the follow up.

Conclusion: Tenofovir treatment in patients with HBV is very effective in terms of suppression of HBV DNA, normalization of ALT and AST, occurrence of liver cirrhosis and HCC.

O-116

Induction of IFN- λ 3 by ADV or TDF in HBV patients

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Background and aim: Clinical significances of *IL-28B* polymorphisms in patients with hepatitis B virus (HBV) infection are controversial while its critical roles were established in chronic hepatitis C.

Methods: Serum IFN- λ 3 was measured in 254 HBV patients by chemiluminescence enzyme immunoassay, which demonstrated little or no cross reactivity between IFN- λ 2 and IFN- λ 3, and clear linearity at typical serum levels. *In vitro* experiments, induction of IFN- λ 3 by nucleos(t)ide analogs was examined in several cell lines originated from skin, lungs, liver, lymphocytes and colon that had potential IFN-

λ s. IFN- λ 3 production was further assessed by real-time PCR and immunohistochemistry.

Results: No differences in serum IFN- λ 3 levels were observed in disease progression, *IL-28B* polymorphisms or HBeAg positivity. Unexpectedly, we found higher serum IFN- λ 3 levels in patients treated with adefovir pivoxil (ADV) or tenofovir disoproxil fumarate (TDF), compared with lamivudine or entecavir without any differences in their clinical backgrounds, which were confirmed by its serial measurement in each patient. *In vitro* experiments showed that ADV or TDF induced IFN- λ 3 only in the colon cancer cell lines in a dose-dependent manner, which were further confirmed by their mRNA up-regulation and positive staining in immunohistochemistry.

Conclusions: We found a novel additional effect of ADV or TDF to induce IFN- λ 3, as well as HBV DNA polymerase inhibition. It is interesting to speculate that orally administered ADV or TDF induce IFN- λ 3 in the gastrointestinal tract, and flow into the liver via portal vein where inhibition of HBV replication might be expected. This study could provide a novel insight in future anti-HBV treatment.

O-117

Mapping HBsAg epitope profiles predictive of seroclearance in genotype A CHB patients on TDF therapy

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Background: Few biomarkers predict HBsAg clearance, which is the ultimate CHB therapy goal. Therapeutic and immune pressure on HBsAg can influence conformation or epitope availability within major antigenic loops. HBsAg epitope profile analysis in a treatment naïve cohort of genotype A CHB patients on TDF; aimed to study if an HBsAg clearance profile (CP) is predictive of HBsAg clearance.

Methods: The HBsAg epitope profile was interrogated using a described 19plex immunoassay. We studied 25 genotype A (HBeAg+) CHB patients from GS-US-174-0103, of whom 14 achieved HBsAg loss, and 11 had no HBsAg response (<0.5 log). Classification with an HBsAg CP (reduced recognition at both loops 1 and 2 epitopes) or an HBsAg non-clearance profile (NCP: no profile change, or reduced at one loop only), was related to HBsAg response on-TDF.

Results: Pre-treatment, 15/25 NCP and 10/25 CP were identified. During treatment, 7 NCPs switched to CPs and achieved HBsAg clearance; while 8 NCPs were maintained (only 2 attained clearance). Of the 10 CPs pre-treatment, 5 were maintained (3 developed clearance), whilst 5 CPs switched to NCP (only 2 achieved clearance). On-treatment analysis to 48 weeks identified 12/25 CP (10 achieved clearance). Only 4/13 NCP achieved clearance. The association

between an HBsAg CP and HBsAg clearance was significant (p-value 0.02, PPV 83 %).

Conclusions: Analysis of the HBsAg epitope profile on-TDF highlighted selection pressure targeting HBsAg before/during clearance, possibly reflecting an emerging anti-HBs response. The development of an HBsAg CP by epitope mapping could be a potential predictive biomarker, and facilitate individualised patient therapy.

O-118

Effectiveness of NAs therapy in real world naive Chinese CHB patients: results from EVOLVE study

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Aims: The main objective of this study is to assess the effectiveness of various nucleos(t)ide analogue (NA) therapies in naive chronic hepatitis B (CHB) patients in real-life practice in China.

Methods: This 5-year prospective, observational study was conducted in China second-tier city hospitals. Patients received entecavir (ETV), lamivudine (LAM), telbivudine (LDT), adefovir (ADV) and combinations of NAs according to physician-patient decision. Virologic, biochemical and serologic responses were assessed following guideline recommendations. Baseline, week 52 and week 96 HBV DNA were tested in central lab. The comparative effectiveness of different treatments on virologic response (VR) was evaluated using logistic regression and propensity score matching. LAM monotherapy throughout 52 weeks, LAM add on ADV during 52 weeks and LAM/ADV de novo combination therapy were grouped together in effectiveness analysis as LAM based group.

Results: 3434 patients were enrolled and 2851 with week 52 HBV DNA results. Week 52 effectiveness results were listed in table 1. Significantly higher proportion of patients receiving ETV achieved VR at Week 52 compared with LAM-based therapy (76.2 versus 59.4 %, p < 0.001). The difference in VR rate between the two groups remained significant after regression adjustment (p < 0.001) and propensity score matching (p < 0.001). During 52 weeks, 3.5 % of patients receiving ETV at baseline modified their initial treatment, primarily due to economic reasons; 7.1 % of patients receiving LAM at baseline modified initial treatment, primarily due to virologic breakthrough and genotypic resistance.

Conclusions: ETV monotherapy was more effective compared with LAM based therapy in a real-world setting in China.

Table 1: Baseline characteristics and Week 52 ± 8 effectiveness results*

Baseline characteristics	ETV N=1818	LAM-based ^a N=630	LDT N=799	ADV N=157	Other de novo combination ^b N=26
Mean age, years (SD)	41 (11.3)	41.3 (12.2)	34.4 (11.2)	41.3 (9.8)	36.6 (10.6)
Male, n (%)	1359 (74.8%)	472 (74.9%)	565 (70.7%)	118 (75.2%)	18 (69.2%)
HBeAg-positive, n (%)	1061 (59.0%)	371 (59.3%)	568 (71.6%)	53 (33.8%)	22 (84.6%)
Mean HBV DNA (log copies/mL)(SD)	7.32 (1.6)	7.30 (1.65)	7.65 (1.52)	6.19 (1.69)	7.70 (1.36)
Cirrhosis, n (%)	432 (23.8%)	161 (25.6%)	97 (12.1%)	57 (36.3%)	4 (15.4%)
Initial treatment modification ^c rate, n (%)	63 (3.5%)	45 (7.1%)	52 (6.5%)	15 (9.6%)	3 (11.5%)
Week 52 effectiveness outcomes					
Virologic response ^d , n/N (%)	1163/1527 (76.2%)	306/515 (59.4%)	434/659 (65.9%)	78/131 (59.5%)	12/19 (63.2%)
HBeAg loss, n/N (%)	313/1068 (29.3%)	139/375 (37.1%)	184/570 (32.3%)	21/54 (38.9%)	5/22 (22.7%)
HBeAg seroconversion, n/N (%)	152/1068 (14.2%)	47/375 (12.5%)	88/570 (15.4%)	6/54 (11.1%)	3/22 (13.6%)
HBsAg loss, n/N (%)	12/1774 (0.7%)	6/622 (1.0%)	5/763 (0.7%)	1/157 (0.6%)	0%
HBsAg seroconversion, n/N (%)	9/1774 (0.5%)	2/622 (0.3%)	3/763 (0.3%)	1/157 (0.6%)	0%
Virologic breakthrough before Week 60, (Kaplan-Meier), %	1.6%	15.1%	8.4%	1.0%	0%
Genotypic resistance before Week 60 (Kaplan-Meier), %	0%	10.8%	5.4%	0.9%	0%

ADV, adefovir; ETV, entecavir; LAM, lamivudine; LDT, telbivudine; SD, standard deviation

*Analysis was based on available blood samples

^aLAM-based: LAM monotherapy through 52 weeks (n=422), LAM add on ADV during 52 weeks (n=16), LAM/ADV de novo combination (n=192)

^bOther de novo combination: LDT+ADV (n=22), ETV+ADV(n=3), ETV+LAM (n=1)

^cInitial treatment modification included dose change, treatment switch, treatment add-on and treatment stop.

^dDefined as HBV DNA <300 copies/mL.

^eResistance tests were performed at treating physicians' discretion.

O-119

Effect of addition AHCC on HBsAg level in patients with CHBeAg negative on long term OAA Treatment

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Background: CHBeAg neg. patients who were on OAA usually relapse HBV DNA after terminate treatment especially if HBsAg still high. Prolong treatment can cause side effect, drug resistant and economic burden to patients.

Objective: To study the effect of HBsAg decline when AHCC was combined with OAA.

Study: CHBeAg neg who were on OAA with HBV DNA undetectable for more than 2 years were enrolled. AHCC 1 g TID was added for 12 months. The HBsAg level was measured every 3 months with chemiluminescence immunoassay, Roches. The rate of HBsAg decline was compared with 6 month level prior to AHCC in the same patients as control.

Result: There were 39 patients (13 females, 26 males) with mean age of 53.69. The average HBsAg level before AHCC treatment was 2458 IU/ml, the level of HBsAg level at pre-treatment, treatment period and post-treatment were 2440.80, 2281 and 2045.70 IU/ml, after adjust with age and sex were 2282 and 2210 IU/ml, by Linea Prediction Test with 95 % confidence, showed statistic significant of HBsAg decline at 12 months ($P < 0.05$). In addition, when we analysis individually, we found 4 patients (10.25 %) who show dramatically drop of HBsAg level after added AHCC.

Conclusion: By overall statistical analysis, AHCC can improve HBsAg decline at months 12, compare with OAA alone and good response in some cases. Large number of subject could be confirmed.

O-120

Characterization of serum HBV RNA in untreated HBeAg-positive chronic hepatitis B patients

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Background: As a DNA virus, the detection of serum HBV RNA has been reported in a few studies, especially in those patients under nucleos(t)ide analogue treatment. However, little is known about its characteristics in HBeAg-positive chronic hepatitis B (CHB) patients before therapy.

Methods: One hundred and nine HBeAg-positive CHB patients without antiviral treatment 6 months prior to enrollment were studied. An efficacious in-house reverse transcription and real time quantitative PCR assay was established and applied for the measurement of serum HBV RNA levels. The lower detection limit was 100 IU/ml.

Results: HBV RNA could be detected in 84.4 % (92/109) of the samples and its median (range) level was 5.3 (3.0–7.1) log IU/ml in RNA-positive samples. The serum DNA and HBsAg levels in RNA-positive patients were found to be significantly higher than those in RNA-negative ones ($P < 0.001$, Table). The correlation coefficients of serum HBV RNA level with DNA, HBsAg levels were 0.522 and 0.468 ($P < 0.001$, Figure), respectively in RNA-positive group. In addition, the proportion of B genotype infection (36/91, 39.6 %) in RNA-positive group was far exceeding than that (1/17, 5.9 %) in RNA-negative group ($P = 0.016$). However, the statistical differences in sex and age between two groups were not revealed.

Conclusions: The finding of serum HBV RNA in the majority of HBeAg-positive CHB patients and its close correlation to serum HBV DNA, HBsAg and genotype call for more investigation in terms of its roles in viral replication, infection and survival.

Key Words Hepatitis B virus; HBV RNA; HBeAg-positive; Chronic hepatitis B; Genotype

Figure.

Correlation analysis between serum HBV RNA, DNA and HBsAg levels in RNA-positive group.

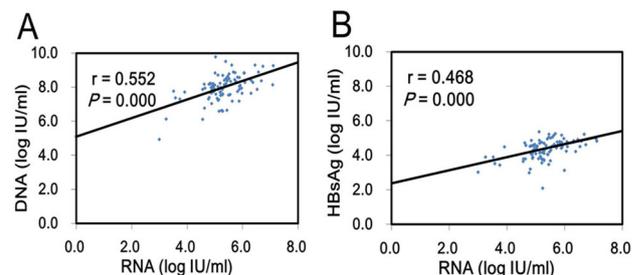


Table.

Comparison of the main demographic and virological characteristics between RNA-positive and RNA-negative patients.

Characteristics	RNA-positive (n=92)	RNA-negative (n=17)	P-value
Male/female (% male)	69/23 (75.0)	14/3 (82.4)	0.721*
Age (years), median (range)	30.0 (18.0–59.0)	33.0 (19.0–55.0)	0.362*
RNA (log IU/ml), median (range)	5.3 (3.0–7.1)	NA ^c	NA
DNA (log IU/ml), median (range)	8.0 (5.0–9.8)	7.3 (4.1–8.4)	0.001*
RNA/DNA (%), median (range)	0.2 (0.0–8.8)	NA ^c	NA
HBsAg (IU/ml), median (range)	27092.0 (124.0–231720.0)	8796.0 (7.0–40396.0)	0.000*
HBV genotype			
B (n=37)	36	1	0.016*
C (n=71)	55	16	
CD recombination (n=1)	1	0	

*Pearson Chi-Square (the case with CD recombination genotype infection was excluded).

^aIndependent sample T-test.

^cNA: not available due to lower than the lower detection limit (100 IU/ml) or undetectable.

O-121

HBV pregenome RNA virus is associated with persistence of HBV infection and viral rebound**Fengmin Lu, Jie Wang, Xiangbo Huang**

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Background: Considering the economic burden and emergence of drug-resistant strains, a predictive factor is needed for monitoring the safe discontinuation of medication. Among HBV serum markers, HBV RNA has recently been suggested to be associated with efficacy and prognosis of chronic hepatitis B (CHB) treatment. However, the characteristic of such HBV RNA and its potential clinical significance is still elusive.

Methods: The dynamic change of serum HBV RNA in CHB patients treated with nucleot(s)ide analogues (NAs) was measured using quantitative real-time RT-PCR. Northern blot, sucrose gradient centrifugation, multiple identification PCR and electron microscopic observation were performed to investigate the origin of serum HBV RNA using cell and transgenic mice HBV replication models.

Results: Through a series of experiments, we firstly confirmed that serum HBV RNA was pregenome RNA (pgRNA) present in HBV viral particles. Such HBV pgRNA virus could be significantly increased when the reverse-transcription of encapsidated pgRNA was inhibited by NA treatment and P protein Y63F mutation. Clinically, we found that the ratio of viral pgRNA to DNA (RNA/DNA) was significantly increased after NA therapy, and higher RNA/DNA ratio at the time point of NA therapy discontinuation was associated with higher risk of HBV DNA rebound.

Conclusions: This study suggests the presence of HBV pgRNA viral particle which is produced by the encapsidated and non- or partially reverse-transcribed pgRNA, and may provide an alternative explanation for the eradication difficulty of HBV infection. Clinically, serum HBV RNA/DNA ratio is a potential predictor for monitoring the safe discontinuation of NA therapy.

O-122

Gene chip detected hepatitis B virus “a” determinant variants**Yanwei Zhong, Qiqi Liu, Shengqi Wang, Hailian Wu**

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Background/aim: This study is to establish gene chip technology and detect hepatitis B virus surface antigen (HBsAg) “a” determinant gene mutation in CHB patients.

Methods: Designed the primer probe of HBsAg common variation sites (s118¸ s120, s126, s133, s145). Amplified target gene using Multiplex Amplification Refractory Mutation System PCR (multi-ARMS PCR), established the tiling chip technology. 40 serum of CHB patients with was detected using this gene chip technology for the 118¸120¸126¸133¸145 sites amino acid and compared to the DNA sequencing method.

Results: Among 40 CHB patients, the gene chip technology detect the wild-type accounted for 3 cases, T118 K and G145A mutations

account for 1 case, T118K and G145R mutations account for 2 cases, P120Q, T126I and G145R mutations account for 2 cases, T126I and G145R mutations account for 2 cases, T126I mutation accounted for 24 cases, M133T mutations account for 2 cases, M133L mutation accounts for 1 case, G145A mutation accounts for 1 case, G145R mutations account for 1 case, 126 sites accounted for 1 case of mixed infection, which consistent with the results of DNA sequencing.

Conclusion: Compared to the classical DNA sequencing method, using gene chip detected HBsAg “a” determinant of variation has more convenient operations, requires less time. It is a fast, accuracy, high-throughput and a more scientific clinical evaluation method.

O-123

Hepatitis B reactivation in CHC Chinese treated with pan-oral DAAs—a prospective study**Cheng Wang^{1,3}, Dong Ji³, Jing Chen¹, Qing Shao³, Fan Li³, Bing Li³, Jia-Liang Liu³, Vanessa Wu¹, Apri Wong¹, Yu-Dong Wang¹, Chris Wong⁴, Xiao-Yong Zhang², Jian Sun², Jin-Lin Hou^{2,3}, Guo-Feng Chen³, George Lau^{1,3}**

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Background and aim: Hepatitis due to hepatitis B virus (HBV) reactivation has been reported in chronic hepatitis C patients (CHC) co-infected with HBV, treated with direct acting antiviral (DAAs) agents. We examine the incidence and predicting factors for “hepatitis” during pan-oral DAAs anti-HCV therapy in an HBV endemic area.

Methods: We prospectively studied 355 consecutive CHC (GT1b/2a/3/6:287/59/7/2) Chinese treated with 8–24 weeks pan-oral DAAs (Solvadi® plus Daklinza®/ Harvoni®/Viekira®:57/291/7). Among them, 10 (2.8 %) were HBsAg positive (all HBeAg negative) and 3 were on anti-HBV nucleos(t)ide analogues (NUCs). Serum samples collected 2–4 weekly during DAAs treatment and then 4–8 weekly till post-treatment week 12, were tested for HBV DNA (Abbott RealTime HBV assay), qHBsAg (Abbott Architect assay) for HBsAg positive patients or those with an increase in serum ALT >2.0 × nadir (“hepatitis”). Precore G1896A, basal core promoter A1762T/G1764A variants were analysed by PCR-pyro-sequencing. Multivariate Cox regression analysis was used to determine risk factors associated with hepatitis.

Results: Eleven (3.1 %) CHC patients suffered from hepatitis during DAAs therapy at week 8 (median, range: 2–12 weeks), with three due to HBV reactivation (anicteric hepatitis/icteric hepatitis/hepatic failure: 2/0/1) and eight due to other causes (all anicteric hepatitis). HBsAg positive patients not treated with anti-HBV NUCs is the most significant factor associated with hepatitis in CHC patients treated with pan-oral DAAs (HR: 12.28, C.I. 3.0–50.6, p = 0.001).

Conclusions: HBsAg positive CHC Chinese treated with pan-oral interferon-free DAAs regimen has an increase risk for hepatitis due to HBV reactivation and pre-emptive anti-HBV nucleos(t)ide analogues need to be considered.

O-124

Clinical relapse after cessation of tenofovir in HBeAg negative patients: presentation and predictor

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Background: Clinical relapse (CR) in HBeAg-negative chronic hepatitis B (CHB) patients occurred in 45 % by 1 year after entecavir (ETV) withdrawal by APASL stopping rule. Of the CR, 26 % occurred within 6 months. Little is known about the CR after stopping tenofovir (TDF) and need to be studied.

Patients and methods: By APASL stopping rule (treatment >2 years, HBV DNA undetectable >1 year), 69 HBeAg-negative patients were followed-up monthly for 3 months and then every 1–3 months after cessation of TDF. Serum HBV DNA was measured by Roche Cobas Amplicor HBV Monitor (limit of detection: 20 IU/ml) and HBsAg by Roche Elecsys® II kit (diagnostic range 0.05–52000 IU/mL).

Results: Of the 69 patients, the 1-year cumulative incidence of CR (ALT > 2X ULN and HBV-DNA > 2000 IU/mL) was 56 %. CR occurred in 26 of the 53 patients who were followed-up >6 months, 61.5 and 88.5 % within 3 and 6 months, respectively. Of the CR, ALT flared >10X ULN in 61.5 %, bilirubin >2 mg/dl in 23.1 % and 3 cirrhotic patients decompensated, much earlier and more severe than those off ETV therapy (Table 1). Higher baseline qHBsAg (Crude OR: 2.957, P = 0.019) and end of treatment qHBsAg (Crude OR: 2.744, P = 0.038) were factors for CR while consolidation >24 months decreased CR (Crude OR: 0.109, P = 0.008).

Conclusions: Clinical relapse occurred much earlier after cessation of TDF than after cessation of ETV therapy. Close monitoring in the first 6 months, especially in cirrhotic patients, is mandatory after cessation of TDF treatment.

O-125

Down-regulation of NTCP in proliferating hepatocytes by p53 and cyclin D1 has clinical significance

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Background: The liver-specific cotransporter polypeptide for the sodium taurocholate (NTCP) was recently identified as a functional receptor for hepatitis B virus (HBV). Previous studies indicated that the expression of NTCP may be associated with the proliferation status of hepatocytes. This study aimed to explore the underlying

mechanism(s) and clinical significance of NTCP down-regulation in proliferating hepatocytes.

Methods: NTCP expression levels and the presence of intrahepatic covalently closed circular DNA (cccDNA) were comparatively analyzed in hepatocellular carcinoma (HCC) tissues. To evaluate the correlation of NTCP expression and cell cycle progression, HCC cell lines were arrested in G0/G1 phase. Transcriptional regulation of the NTCP promoter was detected by using both Luciferase report assays and ChIP-PCR. The impact of NTCP on hepatocytes proliferation was also studied.

Results: NTCP was down-regulated in HCC tumor tissues, and its expression level was significantly higher in cccDNA-positive tissues compared with those cccDNA-negative tissues. NTCP expression maintained at higher levels has statistically significant benefits on post-surgery survival of HCC patients. Forced HCC cells arrested in G0/G1 phase upregulated NTCP mRNA levels. Mechanistically, p53 could suppress NTCP expression via direct binding to the NTCP promoter. In common with p53, cyclin D1 could also transcriptionally down-regulate NTCP expression in a p53-dependent manner. Ectopic NTCP expression could suppress hepatocytes proliferation.

Conclusions: The transcriptional inhibition of NTCP expression during cell cycle progression was mediated by p53 and cyclin D1. NTCP expression level might serve as a novel prognostic predictive biomarker for post-surgery overall survival of HCC patients.

O-126

Angiopoietin-like protein 2, a novel biomarker for liver histology in chronic hepatitis B Patients

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For hepatitis patients who do not meet the treatment criteria recommended by guidelines, therapy decisions depend on hepatic histology. Angiopoietin-like protein 2 (Angptl2) is a mediator of chronic inflammation that contributes to extracellular matrix remodeling. The aim of this study was to explore the predictive value of Angptl2 as a biomarker of liver histology. Three hundred and fifty-one patients with chronic hepatitis B virus (HBV) infection were prospectively enrolled. According to guidelines, anti-HBV treatment was pending in 191 patients. Serum Angptl2 concentrations were detected using the Human ANGPTL2 Assay kit. The histological activity index (HAI) and fibrosis score were assessed according to Ishak criteria. Serum Angptl2 concentration was significantly associated with both HAI ($p < 0.001$, Spearman $\rho = 0.374$) and fibrosis scores ($p < 0.001$, Spearman $\rho = 0.386$) in the therapy-pending group and all 351 patients. Patients with moderate or severe inflammation and fibrosis (HAI > 5 or F > 3) who required urgent anti-HBV therapy exhibited much higher Angptl2 concentration (5.89 ± 2.67 vs. 4.14 ± 1.78 ng/ml, $p < 0.001$). Serum Angptl2 concentration showed areas under the receiver-operating characteristic curve (AUC) of 0.71 for predicting the need for urgent anti-HBV medicine in the therapy-pending group, which is superior to existing non-invasive fibrosis assessments.

Conclusion: Higher serum Angptl2 concentration represents a novel biomarker to independently predict moderate to severe inflammation and fibrosis, which may avoid unnecessary biopsies in up to half of HBV-infected patients with pending therapy decisions.

O-127

Altered expression of STAT1, MX and SOCS3 associated with outcome of therapy in HBV infection**Meifang Han¹, Yong Li¹, Yuanya Zhang¹, Xiaoping Luo², Qin Ning¹**¹Department and institute of Infectious diseases, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China; ²Pediatrics Department, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China**Aim:** To define the relativity between IFN signal pathway and outcome of antiviral treatment, we examined and compared the dynamic change of expression of some IFN signal molecules in both responders and non responders in CHB patients treated with Peg-IFN.**Methods:** In OSST clinical trial, The expression of STAT1, MX and SOCS3 in both peripheral blood mononuclear cells and liver biopsies samples from 54 chronic hepatitis B patients who switching to PEG-IFN from long term ETV treatment were evaluated.**Results:** At week 48 the end of treatment, the proportion of responders achieving HBeAg seroconversion or HBsAg clearance were greater in the Peg-IFN group than that in the ETV group (25.9 % versus 0, $p = 0.005$). Compared with ETV, Peg-IFN altered the expression of IFN signaling molecules STAT1, MX and inhibitory regulator SOCS3 in the PBMCs. Among Peg-IFN treatment group, responders showed the highly expression of STAT1 and MX in PBMCs and in liver compared with non-responders, whereas the non-responders displayed greater expression of SOCS3. Furthermore, for Peg-IFN treated patients, the expression level of STAT1 at week 4 and MX at week 12 in PBMCs correlate with HBsAg decline from baseline to week 48, respectively.**Conclusions:** Our data first in IFN treated patients demonstrated that high expression of STAT1 and MX was positively involved in the strong antiviral response to IFN, whereas inhibitory factor SOCS3 might negatively related with a response to IFN in CHB patients.

O-128

HBV rtA181T and rtM204I mutants originate independently during NAs antiviral therapy**Bin Zhou¹, Hui Dong², Yungang He³, Jian Sun¹, Weirong Jin^{2,4}, Qin Xie⁵, Rong Fan¹, Jinlin Hou¹**¹Department of Infectious Diseases, Nanfang Hospital, Southern Medical University, Guangzhou, China; ²Shanghai-MOST Key Laboratory of Health and Disease Genomics, Chinese National Human Genome Center at Shanghai, Shanghai, China; ³CAS Key Laboratory of Synthetic Biology, Institute of Plant Physiology and Ecology; Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai, China; ⁴Shanghai Shenyou Biotechnology Co., Ltd., Shanghai, China; ⁵Department of Infectious Diseases, Ruijin Hospital, Shanghai Jiaotong University School of Medicine, Shanghai, China

Reverse transcriptase (RT) mutations contribute to hepatitis B virus (HBV) resistance during antiviral therapy with nucleos(t)ide analogs (NAs). However, the composition of the RT quasispecies and their interactions during antiviral treatment have not yet been thoroughly defined. In this report, 10 patients from each of 3 different virological response groups, i.e., complete virological response,

partial virological response and virological breakthrough, were selected from a multicenter trial of Telbivudine treatment. Variations in the drug resistance-related critical RT regions in 107 serial serum samples from the 30 patients were examined by ultra-deep sequencing. A total of 496,577 sequence reads were obtained, with an average sequencing coverage of 4641X per sample. The phylogenies of the quasispecies revealed the independent origins of two critical quasispecies, i.e., the rtA181T and rtM204I mutants. Data analyses and theoretical modeling showed a cooperative-competitive interplay among the quasispecies. In particular, rtM204I mutants compete against other quasispecies, which eventually lead to virological breakthrough. However, in the absence of rtM204I mutants, synergistic growth of the drug-resistant rtA181T mutants with the wild-type quasispecies could drive the composition of the viral population into a state of partial virological response. Dynamical changes of HBV during virological breakthrough development illustrated that it is reasonable to add Adefovir as rescue therapy for treatment of Telbivudine resistant patients and NAs with different pathways would suppress resistant HBV quasispecies respectively.

O-129

Plasma microRNA levels are associated with therapy response in chronic hepatitis B patients**Meike H. van der Ree^{1,2}, Louis Jansen^{1,2}, Karel van Dort², Robert B. Takkenberg¹, Neeltje A. Kootstra², Hendrik W. Reesink^{1,2}**¹Department of Gastroenterology and Hepatology, Academic Medical Center, Amsterdam, The Netherlands; ²Department of Experimental Immunology, Academic Medical Center, Amsterdam, The Netherlands**Aim:** The aim of this study was to identify baseline plasma miRNA-signatures that predict response to peginterferon based therapy in chronic hepatitis B (CHB) patients.**Methods:** We included 86 CHB patients who participated in an investigator-initiated study and completed 48 weeks of peginterferon alfa-2a and adefovir combination therapy, followed by a treatment-free follow-up of 2 years. Plasma levels of 179 miRNAs were determined by qPCR in an identification cohort of 12 HBeAg-positive and 12 HBeAg-negative patients, both consisting of 6 non-responders (NR) and 6 combined-responders (CR) (HBeAg negativity, HBV-DNA <2000 IU/mL, and ALT normalization) of which 3 patients had HBsAg loss. Next, a selection of miRNAs (>1.5-fold difference ($p < 0.05$) and/or >2-fold difference ($p < 0.1$) between study groups in the identification cohort) was measured by qPCR in 86 CHB patients.**Results:** In total, 45 HBeAg-negative and 41 HBeAg-positive patients were included in the study. Both CR ($n = 24$), of which 13 patients achieved HBsAg loss, and NR ($n = 62$) were represented. HBeAg-positive patients had significant higher baseline plasma levels of 10 miRNAs compared to HBeAg-negative patients, whereas miR-301a-3p level ($p = 0.02$) was lower. HBeAg-positive with CR ($n = 14$) had a 1.8-fold higher level of miR-301a-3p ($p < 0.01$), 1.3-fold higher level of miR-146b-5p ($p = 0.03$) and 1.5-fold higher level of miR-143-3p ($p = 0.04$) at baseline compared to NR ($n = 27$). HBeAg-negative patients with HBsAg-loss ($n = 8$) had 2.5-fold higher plasma level of miR-127-3p ($p < 0.01$) and 1.5-fold higher miR-145-5p level ($p = 0.02$) compared to HBeAg-negative patients without HBsAg loss.**Conclusions:** We identified several miRNAs associated with HBeAg status and treatment response in CHB patients.

O-130

HBsAg and HDV RNA reduction with REP 2139-Ca and peg-IFN alpha 2a in chronic HBV/HDV infection.

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Nucleic acid polymers (NAPs) inhibit the release of HBsAg and the NAP REP 2139 can efficiently clear HBsAg from the blood of patients with HBV mono-infection. REP 2139-Ca therapy combined with pegylated interferon alpha-2a is being evaluated in Caucasian patients with HBV/HDV co-infection (NCT02233075).

Patients received REP 2139-Ca once weekly for 15 weeks (500 mg) by 2 h IV infusion, followed by combined therapy for 15 weeks with pegylated interferon alpha-2a (180ug SC qW) with 250 mg REP 2139-Ca. Patients then transition to 33 weeks of pegylated interferon alpha-2a monotherapy. HDV RNA, HBV DNA, HBsAg and anti-HBs are followed every two weeks using standard assays (Robogene RT-PCR, Abbott RealTime HBV, Abbott Architect).

On treatment, observed HBsAg reductions are currently ~5 logs in 6 patients (all <1 IU / ml), ~3 logs in three patients and ~0.5 to 1.5 logs in three patients. HDV RNA is currently undetectable in ten patients (~5 to 8 log reduction from baseline) with ~3 and ~5 log reductions observed in the other two patients. Substantial elevation (389–15,408 mIU/ml) of serum anti-HBs and the development of liver flares were only observed with the onset of exposure to pegylated interferon alpha-2a and was only evident in patients with serum HBsAg <1 IU / ml at the start of immunotherapy.

REP 2139-Ca is able to achieve rapid reductions in serum HBsAg and HDV RNA in Caucasian patients with HBV/HDV infection. REP 2139-Ca may become an important new therapeutic option for patients with chronic HBV/HDV infection.

O-131

Novel mouse model of NASH-driven HCC whose progression depends on TNF- IKK pathway

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We recently identified premalignant HCC progenitor cells (HcPC) in diethylnitrosamine (DEN)-administered mice (Cell 2013). Transplantation of HcPC into MUP-uPA transgenic mice which undergo continuous liver inflammation could progress malignant transformation from HcPC to HCC. To determine whether obesity promotes this process, we fed high-fat diet (HFD) to MUP-uPA mice after HcPC transplantation. As expected, HFD significantly accelerated tumor progression at 5 months after transplantation. Of note, feeding HFD

to MUP-uPA mice resulted in steatohepatitis that resembles the pathology of human NASH, with ballooning degeneration, hepatocyte death and pericellular/bridging fibrosis, and furthermore, long term feeding of HFD (9 months) developed spontaneous HCC without HcPC transplantation. Thus, by combination of these two models, we could separate the effects of NASH on tumor promotion/progression process from the initiation process. Using this method, we assessed the role of TNF, because its expression was significantly elevated in HFD-fed MUP-uPA liver. In spontaneous HCC model, systemic ablation of TNF receptor 1 (TNFR1) by crossing MUP-uPA with TNFR1 $\text{--}/\text{--}$ mice significantly suppressed both NASH and HCC development. To distinguish the effect of TNF on hepatocarcinogenesis from NASH, we isolated HcPCs from DEN-treated TNFR1 $\text{--}/\text{--}$ mice and transplanted them to MUP-uPA mice. Although TNFR1 was deleted only in tumor cells, HFD-enhanced tumor growth was significantly suppressed, suggesting that TNF can directly accelerate tumor progression. In TNFR1-deleted HCC, NF- κ B activation, chemokine induction, and inflammatory cells infiltration were significantly attenuated, and IKK β ablation in HcPC also prevented HFD-enhanced tumor growth. Thus, TNF- IKK -NF- κ B pathway is an important mediator of HCC growth in NASH.

O-132

A tumor suppressor role of microRNA-9 via targeting IGF2BP1 in hepatocellular carcinoma

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Background: MicroRNA-9 (miR-9) dysregulation is implicated in varieties of human malignancies including hepatocellular carcinoma (HCC), but its role remains contradictory.

Methods: We explored the expression and methylation status of miR-9 in HCC samples, and its tumor-related functions in vitro. Bioinformatics analysis, array-based RNA expression profile, and literature retrieval were performed to identify miR-9 targets in HCC. The potential downstream candidates were validated by luciferase reporter assay, real-time quantitative PCR, western blot and enzyme linked immunosorbent assay (ELISA). The clinicopathologic significances of miR-9 target genes were further explored.

Results: miR-9 was frequently down-regulated in primary HCC. Its silencing was largely contributed by a high frequency (42.5 %) of miR-9-1 hypermethylation, which was correlated with bigger tumor size ($P = 0.0234$). In vitro functional studies revealed that miR-9 restoration retarded HCC cell proliferation and migration. IL-6, AP3B1, TC10, ONECUT2, IGF2BP1, MYO1D, and ANXA2 were confirmed to be miR-9 targets in HCC. Among them, ONECUT2, IGF2BP1, and ANXA2 were confirmed to be aberrantly upregulated in HCC. Moreover, upregulation of ONECUT2, IGF2BP1, and IL-6 were significantly associated with poor post-surgery prognosis ($P = 0.0458$, $P = 0.0037$ and $P = 0.0461$, respectively). Mechanistically, miR-9, partially through a functional miR-9/IGF2BP1/AKT&ERK axis, plays a tumor suppressive role in HCC.

Conclusions: Our study suggests that miR-9 functions as a tumor suppressor in HCC progression by inhibiting series of target genes, including the newly validated miR-9/IGF2BP1/AKT&ERK axis, thus providing potential therapeutic targets and novel prognostic biomarkers for HCC patients.

O-133

Regulation of Hes1 expression by the Wnt transcription factor T-cell factor-4

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Background: The Notch effector Hes1 plays a critical role in stemness of cancer stem cells (CSCs). T-cell factor (TCF)-4 is a key transcription factor in Wnt signaling, which is suggested to be linked with Notch signaling. Among our TCF-4 isoforms cloned previously, the TCF-4J and K pair have been characterized based on the presence (K) or absence (J) of SxxSS motif (Exp Cell Res 2010). TCF-4J was highly expressed in poorly differentiated human hepatocellular carcinoma (HCC) tissues, and the TCF-4J-overexpressing HCC cells (J cells) exhibited high tumor-initiating potential, which is one of the features of CSCs (PLoS One 2013). Thus, the **AIM** of this study was to investigate whether the SxxSS motif of TCF-4 was involved in the regulation of Hes1 expression in HCC cells.

Methods: TCF-4K mutants were prepared by site-directed mutagenesis. Sphere formation assay was used to condense CSC-like cells.

Results: Hes1 was strikingly expressed in spheres of J cells and K-mutant cells in both protein and mRNA levels, while its expression level was 70 % inhibited in K cells. Consistently, protein expression levels of Jagged1 and Notch1 were highest in J cells under both attached and floating conditions. The Notch inhibitor DAPT, a γ -secretase inhibitor, clearly decreased the expression levels of Hes1, suggesting that the Notch-Hes1 axis was functional.

Conclusion: Lack of SxxSS motif in TCF-4 robustly upregulated Hes1 expression in HCC cell spheres. The finding strongly suggests that TCF-4 directly regulates Hes1 in HCC spheres, where CSCs abundantly exist.

O-134

A novel blood-based 3-gene signature to predict development of early human hepatocellular carcinoma

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Background/aim: Serum alpha-fetoprotein (AFP) and ultrasonography (US) are used for hepatocellular carcinoma (HCC) screening, but

neither is able to predict the development of HCC. We have previously validated a blood-based 3-gene signature (3-GS) comprising of amphiregulin (AREG), tumor necrosis factor alpha-induced protein 3 (TNFAIP3) and GTPase IMAP Family Member 5 (GIMAP5) to detect HCC in patients with chronic hepatitis B (CHB) with 82.0 % sensitivity and 90.2 % specificity. This is a prospective study to evaluate 3-GS's ability to predict the development of HCC in cirrhotic patients.

Methods: Sixty-six patients with liver cirrhosis (93.9 %/6.1 % Child's A/B) and no previous history of HCC were recruited in the Singapore General Hospital Department of Gastroenterology and Hepatology from March 2011 to August 2014. Six-monthly surveillance was performed with serum AFP and US and blood samples for 3-GS were collected.

Results: Mean age was 60.4 \pm 7.2 years and 62.1 % were males. Aetiology was CHB in 69.7 %. Follow-up was up to 4.6 years (median 2.6 years). Nine (13.6 %) patients developed HCC at a median of 1.0 year after enrolment. 7/1/1 were BCLC 0 or A/B/C respectively. Mean serum AFP at time of HCC diagnosis was 4.7 \pm 2.6 μ g/L. The 3-GS was positive in 5/9 (55.6 %) cases (1/3/1 were BCLC 0/A/B respectively) and pre-dated diagnosis of HCC by 3–12 months (median 8 months) in 4/5 (80 %) cases. The last patient tested positive 2 months after diagnosis of HCC as previous samples were degraded.

Conclusion: This pilot study suggests the 3-GS can predict the development of HCC in patients with cirrhosis by up to 12 months. Thus it can potentially be used to guide intensity of HCC surveillance.

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Subclassification of BCLC stage B hepatocellular carcinoma using up-to-7 criteria and tumor markers

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Because BCLC-B hepatocellular carcinoma (HCC) includes a wide range of tumor burden, subclassification should be needed to predict prognosis precisely. In this study, we retrospectively analyzed BCLC-B HCC patients treated with transarterial chemoembolization (TACE) to determine clinical factors influencing overall survival (OS). Based on the results, subclassification was done using up-to-7 criteria and tumor markers. The subjects were 126 BCLC-B HCC patients who underwent TACE from January 2000 to September 2013 (median follow-up period, 2.8 years) in our institute. Kaplan-Meier curve and Cox proportional-hazards model were used for analyses. In multivariate analysis, des-gamma-carboxy prothrombin (DCP) <150mAU/ml was an only factor contributing to better OS among patients within up-to-7 ($p = 0.015$), whereas alpha-fetoprotein (AFP) >200 ng/ml was an only factor contributing to worse OS among patients beyond up-to-7 ($p = 0.020$). Then, patients were divided into group A (26 patients) within up-to-7 criteria with DCP <150mAU/ml, group C (18 patients) beyond up-to-7 criteria with AFP >200 ng/ml, and group B (other 82 patients). OS differed significantly among the three groups ($p < 0.001$). The survival rates of groups A, B and C were 96, 85 and

65 % at 1 year, 71, 41 and 24 % at 3 years, and 32, 20 and 6 % at 5 years. In conclusion, subclassification using up-to-7 criteria in combination with DCP and AFP may be useful to predict prognosis precisely in patients with BCLC-B HCC. Tumor markers may play different roles to predict prognosis; DCP may serve as a prognostic marker for less progressive HCC, whereas AFP may be of help for progressive HCC.

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Long term outcome of proton beam therapy for treatment-naive patients with hepatocellular carcinoma

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Background and aims: We and others have reported on the excellent efficacy and safety of proton beam radiotherapy (PBT) for hepatocellular carcinoma (HCC). However, these reports were mostly for the recurrent HCC cases, and no study has demonstrated the long term outcome of PBT for naive HCC patients. Therefore, we here analyzed the long-term efficacy and safety of PBT for treatment-naive HCC.

Methods: One hundred eighty four patients who received PBT for naive HCC since 1991 were analyzed.

Results: Ten year local tumor control rate was 87 %. Five-year, 7-year, and 10-year overall survival (OS) rates were 47, 32 and 20 %, respectively, with a median OS of 58 months. Of the 184 cases, 97 cases were operable but preferred PBT rather than hepatic resection. Their 5-year, 7-year, and 10-year OS rates were 55, 44 and 26 %, respectively, with a median OS of 67 months. Cox multivariate analysis revealed the Child Pugh score and ECOG performance status as significant factors affecting the long-term OS: tumor size and the presence of vascular thrombus were not significant. Neither treatment-related death nor complications of grade 3 or more were observed.

Conclusions: PBT demonstrated excellent long-term local tumor control and OS for treatment-naive HCC cases. These results support the role of PBT as a non-invasive, safe yet curative therapeutic option for HCC. PBT is especially good for aged patients, large HCC, or advanced HCC with vascular invasions.

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The effectiveness of cyber-knife therapy for lymph node metastasis from hepatocellular carcinoma

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Background: Cyber-knife one of the stereotactic radiotherapy offers a treatment option for hepatocellular carcinoma (HCC) patients that are not eligible for surgery, transarterial chemoembolization, chemotherapy, or radiofrequency ablation. We have evaluated the efficacy and safety of Cyber-knife for lymph node metastasis from HCC.

Methods: Our first 13 consecutive patients (mean age 69.8 years, with total 30 lymph nodes) receiving Cyber-knife are presented. Mean radiation dose was 41.6 Gy delivered over 8–10 fractions. The evaluation was made by enhanced CT after 3–4 months from radiation.

Results: The mean observational period was 389 days. Twelve patients were male and 10 patients were positive for HCV. All patients were already treated with sorafenib. The mean diameter of lymph nodules was 54.3 mm. The distribution of each lymph nodes metastasis was as follows: abdominal lymph nodes 25, hilar lymph nodes 2, and cervical lymph nodes 3. Overall response rate (CR + PR) was 100 % (30/30 nodules). There were no severe adverse events which exceeded CTCAE grade 2.

Conclusion: Our preliminary results showed that Cyber-knife is a safe and effective local treatment especially for metastatic lymph node HCC.

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Regional differences in transarterial chemoembolization (TACE) use in HCC: OPTIMIS Interim analysis

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Background: Transcatheter arterial embolization (TACE) is recommended treatment for patients with intermediate-stage hepatocellular carcinoma (IHCC) Barcelona Clinic Liver Cancer [BCLC] stage B). Several retrospective studies indicated continuation of TACE after TACE refractoriness/failure may be harmful to the patient.

Method: OPTIMIS, a prospective, observational study of patients treated with TACE at study enrollment, with or without sorafenib after TACE, will enroll 1650 patients with HCC (BCLC stage B or higher). We performed an interim analysis of patient and TACE treatment characteristics after 500 patients were observed for >6 months. TACE ineligibility was defined using international guidelines/consensus and Japanese Society of Hepatology (JSH) guidelines; analyses were conducted using each definition.

Results: Of 475 patients who received TACE, the majority were from Asian countries/region (68.4 %) including Japan (n = 91), China (n = 80), Asia excluding Japan/China (n = 148); 156 patients were from Europe/Canada. Overall, 24.3 % of patients received sorafenib; however, this percentage varied across countries (range 13.2–40.0 %). At time of initial TACE, overall 54.5 % of patients were not recommended for TACE according to international guidelines/consensus, whereas 22.3 % were not recommended according to JSH guidelines. Median time to TACE ineligibility varied from 42.0 days (n = 5) in China to 127 days (n = 17) in Japan.

Conclusion: In real life practice, there were significant regional differences in TACE treatment, patient selection, and deviations from guidelines. Findings reveal educational needs and standardization of TACE procedures.

Table. Patient characteristics and TACE treatment information

	EUROPE/ CANADA N=156	ASIA (Excl. China/JPN) N=162	JAPAN N=91	CHINA N=80	Total N=489
Patient Characteristics at inclusion visit (prior to TACE)					
Patients treated with TACE, n (%)	148 (94.9)	156 (96.3)	91 (100.0)	80 (100.0)	475 (97.1)
Mean age (SD), y	65.9 (11.0)	63.7 (9.9)	72.9 (9.2)	52.9 (11.7)	64.3 (12.1)
Range	22–90	37–86	50–95	18–77	18–95
Male, n (%)	120 (81.1)	117 (75.0)	68 (74.7)	70 (87.5)	375 (78.9)
Etiology, n (%)					
Hepatitis B	13 (8.8)	68 (43.6)	11 (12.1)	56 (70.0)	148 (31.2)
Hepatitis C	44 (29.7)	51 (32.7)	50 (54.9)	1 (1.3)	146 (30.7)
Alcohol	70 (47.3)	34 (21.8)	20 (22.0)	8 (10.0)	132 (27.8)
Patients treated with sorafenib, n (%)	30 (19.2)	45 (27.8)	12 (13.2)	32 (40.0)	119 (24.3)
BCLC stage, n (%)					
B	111 (75.0)	115 (73.7)	86 (94.5)	27 (33.8)	339 (71.4)
C	21 (14.2)	34 (21.8)	4 (4.4)	52 (65.0)	111 (23.4)
D	1 (0.7)	2 (1.3)	0	1 (1.3)	4 (0.8)
Child-Pugh score, n (%)^b					
A (5–6 points)	94 (63.5)	120 (76.9)	67 (73.6)	66 (82.5)	347 (73.1)
B (7–9 points)	33 (22.3)	31 (19.9)	22 (24.2)	12 (15.0)	98 (20.6)
C (10–15 points)	0	2 (1.3)	0	1 (1.3)	3 (0.6)
Vascular invasion, n (%)					
Portal vein thrombosis	5 (3.4)	9 (5.8)	1 (1.1)	25 (31.3)	40 (8.4)
Hepatic vein invasion	3 (2.0)	3 (1.9)	1 (1.1)	2 (2.5)	9 (1.9)
Longest diameter of tumor (mm)					
Median (range)	45 (11–163)	52 (10–158)	27 (10–180)	59 (13–159)	45 (10–180)
Embolization Agent, n (%)					
Lipiodol	73 (49.3)	124 (79.5)	79 (86.8)	73 (91.3)	349 (73.5)
DC-Beads	51 (34.5)	13 (8.3)	17 (18.7)	0	81 (17.1)
Condition not indicated for TACE at initial TACE, n (%)					
Overall	77 (52.0)	81 (51.9)	32 (35.2)	69 (86.3)	259 (54.5)
ECOG PS ≥ 1	34 (23.0)	57 (36.5)	11 (12.1)	38 (47.5)	140 (29.5)
Child-Pugh B/C	32 (21.6)	31 (19.9)	22 (24.2)	13 (16.3)	98 (20.6)
Vascular invasion	7 (4.7)	12 (7.7)	2 (2.2)	27 (33.8)	48 (10.1)
Extrahepatic spread	3 (2.0)	11 (7.1)	0	15 (18.8)	29 (6.1)
Common conditions not indicated for TACE at initial TACE according to JSH, n (%)					
Overall	29 (19.6)	38 (24.4)	3 (3.3)	36 (45.0)	106 (22.3)
Vascular invasion	7 (4.7)	12 (7.7)	2 (2.2)	27 (33.8)	48 (10.1)
Extrahepatic spread	3 (2.0)	11 (7.1)	0	15 (18.8)	29 (6.1)
Active cardiovascular disease	11 (7.4)	8 (5.1)	1 (1.1)	0	20 (4.2)
Time to TACE ineligibility, days					
Median (range)	79.5 (27–605)	109.5 (31–237)	127.0 (20–711)	42.0 (34–77)	92.0 (20–711)
Number of patients	n=20	n=20	n=17	n=5	n=62

^aMissing values included 21 patients, n=15 from Europe, n=5 from Asia, and n=1 from Japan; ^bMissing values included 27 patients, n=21 from Europe, n=3 from Asia, n=2 from Japan, and n=1 from China.

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Practice patterns, sorafenib dosing and safety in Asian hepatocellular carcinoma patients in GIDEON

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Background: To evaluate practice patterns, dosing, and safety of sorafenib in Asian countries from the GIDEON study.

Method: GIDEON, a global, prospective, nonrandomized, observational study, evaluated the use of sorafenib in patients with unresectable hepatocellular carcinoma. Over 3300 patients from 39 countries were enrolled. Treatment history, and patient and disease characteristics were collected at study entry; outcomes data and adverse events (AEs) were collected during follow-up.

Results: Differences were observed across countries in patients' conditions/characteristics, and treatment patterns, including sorafenib dosing. The majority of patients in Asian countries had Hepatitis B, whereas Hepatitis C was more common in Japan. Japanese patients were older, tended to be diagnosed at an earlier-stage disease and had the longest time from initial diagnosis to the start of sorafenib. Prior locoregional therapy was more common in Asia versus the overall population. Sorafenib daily-dose was lower in Japan and the US. AE profiles were similar across

regions regardless of the given doses and the majority of AEs were grade 1/2; the most common were hand-foot skin reaction and diarrhea. Rates of overall and sorafenib-related AEs were highest in Japan, while death on treatment was low. Reasons for treatment discontinuation varied across countries. Discontinuations due to AEs and disease progression were highest in Japan and loss to follow up was lowest.

Conclusion: These results highlight the global heterogeneity of HCC patient characteristics and treatment patterns even within Asian countries, and indicate the potential influence of these differences on outcomes across countries.

Table. Summary of Patient Demographic, Disease Characteristics, Safety, and Efficacy (Safety Population)

Characteristic	Overall N=3202	Asia Pacific n=928	Korea n=482	China n=331	Japan n=508
Median age, y (range)	62 (15-98)	54 (19-87)	55 (22-87)	50 (19-85)	68.8 (23-90)
HBV/HCV, %	36.5/32.9	82.3/5.0	81.1/5.4	89.4/1.5	24.2/53.1
BCLC stage at initial diagnosis/ study entry, %^a					
A	21.6/7.1	9.1/2.8	6.8/0.8	10.0/5.4	43.7/6.5
B	19.7/19.8	15.8/10.2	11.2/4.8	23.0/17.5	20.3/31.9
C	30.1/52.0	37.6/61.1	34.0/60.8	43.5/61.9	17.7/54.7
D	2.8/5.4	2.6/5.0	1.7/4.6	3.0/5.1	0.8/1.8
Child-Pugh at start of sorafenib, %^a					
A	61.5	63.6	56.8	74.3	85.0
B	20.8	18.6	21.8	14.5	11.4
C	2.3	1.7	1.2	0.6	0
MVI, %	22.2	25.5	25.7	22.4	18.9
EHS, %	39.7	53.9	66.0	40.5	44.1
Median time from initial diagnosis to sorafenib, months	3.88	2.56	3.53	2.04	24.10
Median daily sorafenib dose, mg	688.0	800.0	669.5	800.0	419.0
Discontinuation of sorafenib, %					
Progression, recurrence, or relapse of HCC	24.1	18.9	30.7	2.1	36.9
AE or toxicity	14.8	9.3	12.8	3.3	26.5
Lost to follow-up	10.0	19.6	13.8	26.3	0.2
Previous treatment, %					
Surgery	21.1	24.2	19.5	33.5	43.3
Locoregional therapy	57.5	67.2	68.3	73.1	84.4
TACE	47.2	60.3	60.6	66.8	71.3
Systemic therapy	5.2	5.0	7.1	2.7	11.6
OS ^b , months	10.8 ^a	9.7 ^a	8.4 ^a	10.6 ^f	14.5 ^e
TTP ^b , months	4.8 ^a	3.8 ^a	2.5 ^a	9.0 ^f	3.4 ^e
Time from initial diagnosis to death ^b , months	25.5 ^a	20.9 ^a	24.8 ^a	18.6 ^f	79.5 ^e
AEs, %					
All grades/sorafenib-related	85.3/66.0	70.0/48.7	82.2/60.8	50.5/28.7	94.9/87.6
Grade 3 or 4 (all/sorafenib-related)	31.7/23.6	20.5/12.2	29.9/17.0	6.0/3.6	43.9/37.4
Serious (all/sorafenib-related)	43.3/9.3	33.6/3.4	36.9/4.4	23.3/0.3	41.1/17.7
Death on treatment, % ^b	23.7	19.1	48.1	50.2	15.2

^aData does not include patients who were not evaluable or missing; ^bIntention-to-treat population; n=3213; ^cn=955; ^dn=490; ^en=338; ^fn=500; ^ghappening up to 30 days after last sorafenib dose. AEs=adverse events; BCLC=Barcelona Clinic Liver Cancer Staging; EHS=extrahepatic spread; HBV=hepatitis B virus; HCV=hepatitis C virus; MVI=microvascular invasion; OS=overall survival; TACE=transarterial chemoembolization; TTP=time to progression.

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Prognostic factors and predictors of sorafenib response in patients with hepatocellular carcinoma

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Background: To assist in the design of future studies in HCC, we assessed prognostic factors for potential use in stratification, and predictors of sorafenib response through an exploratory pooled analysis of patients with advanced HCC in the phase 3 trials, SHARP (n = 602) and Asia-Pacific (n = 226).

Methods: Sorafenib showed a significant overall survival (OS) benefit over placebo in both studies. To identify prognostic factors for

OS, exploratory univariate and multivariate analyses were performed for baseline variables by Cox proportional hazards model. Hazard ratios (HR) and median OS were evaluated across pooled subgroups. To assess consistency of sorafenib benefit, the interaction term between treatment and placebo for each subgroup was evaluated using Cox proportional hazards model.

Results: Of 827 patients (448 sorafenib; 379 placebo) analyzed, OS prognostic factors were BCLC stage, tumor burden, tumor size, ECOG PS, alpha-fetoprotein, ALBI grade, and NLR in both arms; race and etiology were not prognostic. Sorafenib OS benefit was consistently observed across all subgroups including race, HBV status, and macroscopic vascular invasion, with numerically lower HRs observed for patients without EHS, tumor burden, with HCV, and lower NLR. Significantly greater OS benefit of sorafenib versus placebo was observed in patients with versus without HCV (HR 0.47 vs 0.81; $p = 0.035$).

Conclusions: Identified prognostic factors predicted poor OS, but not less benefit from sorafenib. Race and etiology do not have prognostic value. However, HCV etiology predicts a greater sorafenib benefit. These findings should be considered when designing future clinical trials in HCC.

Overall Survival According to Prognostic Factors and Prediction of Response ^a						
Baseline covariates	N (PBO;SOR ^b)	Events	Median OS(mos) PBO SOR		HR (SOR/PBO) [95% CI]	Treatment Interaction P-value ^c
Race						
Asian	274 (100;174)	203	4.8	6.7	0.78 [0.59-1.04]	0.3664
Non-Asian	553 (279;274)	344	7.9	11.4	0.67 [0.54-0.82]	
ECOG PS						
0	373 (183;190)	224	9.8	13.0	0.71 [0.54-0.92]	0.465
1 and 2	454 (196;258)	323	4.8	7.4	0.67 [0.54-0.84]	
BCLC stage						
Stage B	113 (55;58)	58	13.4	15.1	0.78 [0.46-1.32]	0.431
Stage C	714 (324;390)	489	6.0	8.5	0.69 [0.57-0.82]	
Tumor burden (presence or absence of vascular invasion, extrahepatic spread or both)						
Absent	229 (107;122)	112	10.6	15.7	0.53 [0.37-0.78]	0.081
Present	598 (272;326)	435	5.6	7.3	0.77 [0.64-0.93]	
MVI						
No	515 (230;285)	294	10.0	12.7	0.70 [0.56-0.88]	0.947
Yes	310 (148;162)	252	4.5	6.0	0.69 [0.53-0.89]	
EHS						
No	365 (178;187)	218	7.2	13.8	0.55 [0.42-0.72]	0.015
Yes	462 (201;261)	329	7.0	7.3	0.84 [0.67-1.05]	
MVI without EHS						
No	689 (307;382)	440	8.5	9.5	0.74 [0.62-0.90]	0.283
Yes	136 (71;65)	106	4.5	6.8	0.53 [0.35-0.80]	
Number of target lesions						
1	208 (99;109)	130	7.0	8.8	0.73 [0.51-1.05]	0.078
2	165 (79;86)	99	9.3	11.8	0.59 [0.40-0.89]	
3	147 (70;77)	91	5.5	11.5	0.49 [0.32-0.75]	
>3	295 (130;165)	218	6.4	7.3	0.85 [0.65-1.12]	
Maximum baseline target lesion size						
< 6 cm	375 (172;203)	213	10.0	14.0	0.61 [0.46-0.80]	0.203
≥ 6 cm	440 (206;234)	325	5.2	7.3	0.75 [0.60-0.94]	
Hepatitis B						
No	540 (266;674)	349	7.4	9.9	0.69 [0.55-0.85]	0.516
Yes	236 (90;146)	172	4.8	6.5	0.78 [0.57-1.06]	
Hepatitis C						
No	568 (265;303)	398	6.8	7.6	0.81 [0.66-0.99]	0.035
Yes	199 (88;111)	120	7.9	14.0	0.47 [0.32-0.69]	
Alcohol Use						
No	636 (287;349)	419	6.5	9.4	0.67 [0.55-0.81]	0.361
Yes	191 (92;99)	128	8.5	9.5	0.82 [0.58-1.17]	
Albumin (median=3.96 g/dL)						
≤ median	355 (143;212)	277	4.1	6.6	0.64 [0.50-0.82]	0.943
> median	472 (236;236)	270	9.8	13.6	0.63 [0.49-0.80]	
Total bilirubin (median=0.77 mg/dL)						
≤ median	473 (214;259)	287	9.2	10.7	0.70 [0.56-0.89]	0.934
> median	354 (165;189)	260	4.9	6.7	0.72 [0.56-0.93]	
ALBI grade^d						
1	433 (214;219)	247	9.9	14.0	0.61 [0.48-0.79]	0.545
2	386 (159;227)	293	4.4	6.4	0.70 [0.55-0.89]	
3	8 (6;2)	7	1.8	0	0.85 [0.08-9.01]	
NLR^e (median=3.08)						
≤ median	430 (192;238)	242	9.8	13.1	0.59 [0.46-0.77]	0.050
> median	391 (184;207)	301	5.0	5.7	0.84 [0.66-1.05]	
Baseline AFP (ng/dL)						
≤200	426 (198;228)	241	9.5	12.5	0.69 [0.53-0.89]	0.877
>200	362 (167;195)	286	5.1	6.5	0.72 [0.56-0.91]	
≤400	471 (216;255)	276	8.8	11.9	0.70 [0.55-0.89]	
>400	317 (149;168)	251	4.9	6.1	0.73 [0.57-0.94]	

^aITT population; ^bSorafenib group 1 patient with BCLC stage D was excluded; ^cWald test for individual effects (p-value); ^dALBI grade, $(\log_{10}[\text{bilirubin} (\mu\text{mol/L})] * 0.66 + [\text{albumin} (\text{g/L}) * (-0.085)])$; ^eNLR, neutrophil absolute count/leucocyte absolute count.

ALBI=albumin bilirubin indicator; BCLC=Barcelona Clinic Liver Cancer; ECOG PS=Eastern Cooperative Oncology Group Performance Score; EHS=extrahepatic spread; MVI=macroscopic vascular invasion; NLR=neutrophil leukocyte ratio; OS=overall survival; PBO=placebo; SOR=sorafenib.

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The role of the phosphorylated RXR α on diethylnitrosamine-induced liver tumorigenesis in mice

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We have reported that a malfunction of RXR α due to aberrant phosphorylation at serine 260 by MAPK is associated with the development of hepatocellular carcinoma (HCC). The phosphorylated RXR α impairs normal receptor functions of either RXR or RAR in a dominant-negative manner. Besides, these impaired receptor functions result in the downregulation of their target gene expression, and inducing hepatocarcinogenesis through the promotion of cell proliferation or the inhibition of apoptosis, which suggesting that phosphorylated RXR α plays a crucial role in the development of HCC. However, these findings were revealed primarily using HCC cell lines that express the phosphorylated-RXR α (p-RXR α) proteins and it still remains unclear whether p-RXR α may impact in the process of hepatocarcinogenesis in vivo. Therefore, in order to investigate the biological functions of the p-RXR α in vivo, we generated doxycycline-inducible transgenic mice that overexpress phosphomimic mutant of RXR α (T82D/S260D). We found that the development of liver tumors induced by liver carcinogen diethylnitrosamine (DEN) was enhanced in the transgenic mice that overexpress T82D/S260D protein in the liver. Besides, the increased liver tumors observed in the transgenic mice resulted from the promotion of cell cycle progression. Interestingly, expression of β -catenin protein and its target genes, *Cyclin D1* and *c-Myc*, were increased in transgenic liver tumors, although the expression of retinoid-related genes, such as RAR β , p21 and p27, were not altered compared with control mice. These results suggest that the phospho-modification of RXR α may promote the development of DEN-induced liver tumors through the activation of β -catenin signaling pathways in mice.

O-142

Transcription factor RFX5 is a transcriptional activator of TPP1 gene in hepatocellular carcinoma

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Background: Regulatory factor X-5 (RFX5) is a classical transcriptional regulator of MHCII genes. But, we found that RFX5 could transactivate non-MHCII genes, such as peptidase 1 (TPP1), but not MHCII genes in HCC. Hence, this study aims to prove that RFX5 is a direct positive transcriptional regulator of TPP1 in HCC.

Methods: Transcriptional target genes of RFX5 were identified using ChIP-seq data of RFX5 from ENCODE, and then validated using

ChIP-PCR. Gene expression data was acquired from the RNAseq data of liver hepatocellular carcinoma (LIHC) in TCGA, and then validated using real-time PCR. Lentivirus expressing RFX5 specific shRNAs and retrovirus expressing RFX5 ORF were used for modulating the expression of RFX5. The promoter transcriptional activity were determined by luciferase assay.

Results: RFX5 overexpressed in HCC compared to non-tumor tissues in two independent HCC cohorts and failed to induce MHCII expression in HCC tissues and cell lines. RFX5 was found to directly bind to TPP1 promoter, which containing a highly conserved RFX5 binding motif (X1 box), by ChIP assay in HCC cell lines. Furthermore, RFX5 could enhance the transcriptional activity of TPP1 promoter, and modulating the expression of RFX5 in HCC cell lines could subsequently alter TPP1 expression level. Most importantly, the mRNA expression level of TPP1 was elevated in HCC and tightly correlated with that of RFX5, and linked to bad prognosis of HCC patients.

Conclusions: RFX5 overexpressed in HCC and transactivated non-MHCII gene, TPP1, which lead to the overexpression of TPP1 and have potential biological implications in HCC.

O-143

CXCL2 plays a tumor suppressing role through recruiting N1 neutrophils in hepatocellular carcinoma

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Background: Currently, it is known that dysregulation of immune system contributed to hepatocellular carcinoma (HCC). Chemokine ligand 2 (CXCL2) could recruit neutrophils to cause inflammation and was closely related to kinds of tumors. This study aimed to explore the underlying mechanism(s) of CXCL2 in HCC.

Methods: Array-based comparative genomic hybridization (aCGH) analysis was used to analyzed the copy number variant of genes in HCC. Expression of CXCL2 was detected by real-time PCR. The effects of CXCL2 on HCC cell proliferation and xenograft tumor formation were evaluated by MTT assay and subcutaneous inoculation in nude and beige-SCID mice which excluding the function of B, T and NK cells. Amount and frequency of N1 neutrophils in peripheral blood and tumor tissues of xenograft mice was detected by flow cytometry. The tumor suppressing N1 neutrophils were characterized by cell surface marker CD11b + Ly6G + Ly6C+.

Results: CXCL2 was deleted in 40 % (10/25) HCC tumor tissues. Its expression level was significantly down-regulated in HCC tumor tissues compared with adjacent non-tumor tissues. CXCL2 had no effect on HCC cell proliferation in vitro, due to the absence of its receptors CXCR1 and CXCR2 on HCC cell membrane. Notably, CXCL2 could suppress tumor formation in nude mice and beige-SCID mice, respectively. Flow cytometry analysis indicated that amount and frequency of N1 neutrophils in CXCL2 group was significant higher than control group.

Conclusions: CXCL2 was down-regulated in HCC tumor tissues. Our data indicated it could act as a tumor suppressor through recruiting N1 neutrophils which subsequently activated anti-tumor immune response.

O-144

DPP4 inhibitor suppressed tumor progression and Nrf expression in a mouse model of NASH-related HCC

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Background and aims: Glucose metabolite is associated with tumor progression through Nrf, a transcription factor regulating re-programming of glucose metabolism. Dipeptidyl peptidase 4 inhibitor (DPP4i) improves glucose metabolism in patients with non-alcoholic steatohepatitis (NASH). We aimed to investigate effects of DPP4i on hepatocellular carcinoma (HCC) and Nrf in a mouse model of NASH.

Material and methods: A mouse model of NASH-related HCC (STAM mice) was employed. Eight-week old mice were administered with either DPP4i (sitagliptin 30 mg/kg/day: DPP4i group; n = 8) or distilled water (CON group; n = 8) for 10 weeks. The incidence, number, and volume of HCC were evaluated by contrast-enhanced computed tomography. Nuclear expression of Nrf was evaluated by immunostaining and quantified by Image J.

Results: Nonalcoholic fatty liver disease activity score were significantly lower in the DPP4i group than in the CON group (3.4 ± 1.2 vs. 5.5 ± 1.5 , $P < 0.05$). No significant difference was seen in the incidence of HCC between the 2 groups. While, the number and volume of HCC were significantly lower in the DPP4i group than in the CON group (1.6 ± 1.8 vs. 4.1 ± 1.8 /liver, $P < 0.01$, 11.6 ± 20.7 vs. 36.6 ± 70.5 mm³/tumor, $P < 0.05$). In immunostaining analysis, nuclear expression of Nrf in HCC was significantly lower in the DPP4i group than in the CON group (286 ± 36 vs. 1751 ± 295 arbitrary unit; $P < 0.01$).

Conclusion: We demonstrated that DPP4i suppressed HCC progression and Nrf expression in a mouse model of NASH. Thus, DPP4i may suppress progression of NASH-related HCC via reprogramming of glucose metabolism.

O-145

DC-based immunotherapy with T cell immunoglobulin and mucin protein 3 blockade in a murine HCC model

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HCC is usually associated with cirrhosis and often recurs even after complete treatment of the tumors in the remaining part of the cirrhotic liver. Immunotherapy may be a new treatment with few side effect and several clinical trials have been performed for HCC treatment. T cell immunoglobulin and mucin protein 3 (TIM-3) has been identified as a marker of immunosuppressive state. Interaction of Tim-3 with its ligand, galectin 9, triggers cell death in activated T cells. In this study, we evaluated the efficacy of the combination of DC vaccine and anti-TIM-3. In protection model, mice were injected with

DCs or/and anti-TIM-3 before the murine HCC tumor cell challenge. 50 % of mice treated with both DCs and anti-TIM-3 rejected tumor challenge. In therapeutic model, tumor-bearing mice were inoculated with DCs or/and anti-TIM-3. (DCs + anti-TIM-3, $265.3 \pm 53.09 \text{ mm}^2$ vs control, $535.1 \pm 44.47 \text{ mm}^2$ on day 30, $P = 0.0046$). To investigate induction of tumor-specific immune responses, we stimulated splenocytes of DCs or/and anti-TIM-3 treated mice twice weekly by DCs in vitro. High tumor-specific immune response was detected when splenocytes of mice treated with DCs and anti-TIM-3 were used as effector cells in interferon- γ enzyme-linked immunospot assay. In addition, incubation with anti-TIM-3 enhanced expression of IL-12p70, and cell surface expression of maturation markers in bone marrow-derived DCs. Our findings suggest that anti-TIM-3 enhanced the maturation of DCs, and Th1-type antitumor immune responses induced by DC vaccine.

O-146

Aspartate- β -hydroxylase generates epitope-specific T cell responses in hepatocellular carcinoma

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Hepatocellular carcinoma (HCC) has a poor prognosis due to a high recurrence rate and lack of effective systemic therapy. Aspartate- β -hydroxylase (ASPH) is a highly conserved transmembrane protein overexpressed in HCC and promotes malignant characteristics. The aim of this study is to define the epitope specific components required for a peptide based candidate vaccine for HCC. Monocyte-derived dendritic cells (DCs) generated from the peripheral blood mononuclear cells (PBMCs) of HCC patients were loaded with ASPH protein. Helper CD4+ T cells and CD8+ cytotoxic T lymphocytes (CTLs) were co-incubated with the DCs; T cell activation was evaluated by flow cytometric analysis. Immunoinformatics tools were used to predict HLA class I- and class II-restricted ASPH sequences, and the corresponding peptides were synthesized. The immunogenicity of each peptide in cultures of human PBMCs was determined by IFN- γ ELISpot assay. ASPH protein-loaded DCs activated both CD4+ and CD8+ T cells contained within the PBMC population derived from HCC patients. Furthermore, the predicted HLA class I- and class II-restricted ASPH peptides were significantly immunogenic. Both HLA class I- and class II-restricted peptides derived from ASPH induced T cell activation in HCC. These results suggest that ASPH protein and related peptides were highly immunogenic in patients with HCC and produce the type of cellular immune responses required for generation of anti-tumor activity.

O-147

Contribution of copper to HIF-1 α activation in the process of hepatocarcinogenesis

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Background: Hypoxia-inducible factor-1 α (HIF-1 α) plays important roles in carcinogenesis. However, the relationship between HIF-1 α and trace elements, including copper (Cu) and zinc (Zn), remains unclear in the process of hepatocarcinogenesis.

Methods: Nine patients with chronic hepatitis (CH), 5 patients with liver cirrhosis (LC), and 12 patients with hepatocellular carcinoma (HCC) were enrolled in this study. Serum HIF-1 α levels were determined using a commercially available ELISA kit. Hepatic HIF-1 α expression was evaluated using an immunohistochemical procedure.

Results: Serum HIF-1 α levels in each category were $5.09 \pm 1.22 \text{ ng/ml}$ in CH, $5.22 \pm 1.77 \text{ ng/ml}$ in LC and $6.47 \pm 1.57 \text{ ng/ml}$ in HCC, indicating that HCC patients had significantly higher serum HIF-1 α level than CH patients ($p = 0.0344$). Serum Cu level in patients with HCC was significantly higher than those in patients with CH and LC ($137 \pm 24 \text{ }\mu\text{g/dl}$ vs $108 \pm 15 \text{ }\mu\text{g/dl}$, $114 \pm 24 \text{ }\mu\text{g/dl}$, $p = 0.0030$, $p = 0.0471$). Interestingly, positive correlation was observed between serum HIF-1 α and Cu levels in the enrolled patients ($r = 0.425$, $p = 0.0137$). Serum Zn levels were decreased in proportion to hepatic fibrosis. However, no significant differences in serum Zn levels were found between patients with LC and patients with HCC. Two of 5 patients with HCC had hepatic HIF-1 α expression, while none of patients with CH and patients with LC did. Most of tumor cells adjunct to necrotic area in these HCC patients were stained with HIF-1 α .

Conclusion: These data suggest that activation of HIF-1 α deriving from Cu accumulation in the liver may lead to hepatocarcinogenesis.

O-148

Significance of the up-regulation of connective tissue growth factor in hepatocellular carcinoma

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Background and aim: Connective tissue growth factor (CTGF) is a multifunctional molecule related with liver fibrosis. We analyzed its roles in hepatocellular carcinoma (HCC).

Methods/results: We used hepatocyte-specific Kras-mutated mice (KrasG12D mice) for a HCC mouse model, generated by crossing mice carrying LSL-KrasG12D allele and Alb-Cre mice. All KrasG12D mice developed liver tumors after 9 months. Similar to most human HCCs, Ras pathway was constitutively activated in tumors. CTGF was also up-regulated in tumor area.

In vitro experiments revealed CTGF up-regulation by EGF-driven Ras-activation in Huh7 cells (Kras wild) and down-regulation by the inhibition of Ras/Mek/Erk pathway in HepG2 cells (Kras mutant). Gene set enrichment analysis using 225 HCC patients' data in NCI data base also showed a positive correlation between CTGF expression levels and activity of Ras/Mek/Erk pathway.

To analyze the significance of CTGF in HCC, we generated hepatocyte-specific CTGF-deficient KrasG12D mice by mating KrasG12D mice and CTGF-floxed mice, and compared with KrasG12D littermates in 8 month. Consequently, CTGF-deficient KrasG12D mice revealed decreased tumor number, diameter, and liver/body weight ratio. Histologically, CTGF-deficient KrasG12D mice showed better tumor differentiation.

Finally, we examined CTGF expression levels in 92 human resected HCCs under IRB approval. CTGF was up-regulated in HCCs compared with non-tumor area. CTGF overexpression was associated with the factors concerning the malignant characteristics of HCC, such as higher PIVKA-II, portal invasion rate, and malignant macroscopic classification. **Conclusion:** CTGF is up-regulated through Ras/Mek/Erk pathway in HCC and promotes the progression of HCC. CTGF could be a novel therapeutic target against HCC.

O-149

MAP promotes HCC and metastasis through the mTORC1 and β -catenin signaling pathways

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Background and aims: Mitosis-associated protein (MAP) was upregulated in some types of human cancers. However, its biological implication and clinical significance have not yet clarified in human cancer, especially HCC.

Methods: Tumorigenicity and metastatic ability were examined in orthotopic animal model. Cell cycle progression and downstream signaling pathway were determined by analyses of flow cytometric activated cell sorting (FACS) and proteome kinase profiling, respectively. miRNA microarray was used to analyze the differentially expressed miRNAs, and the results were validated by qPCR.

Results: MAP expression was strongly detected in early and advanced tumors by gene expression profiling and immunohistochemical staining analyses. MAP expression was mainly associated with G2/M phase of the cell cycle progression, which was associated with the regulations of Cdc2 and Cdc25C, which might contribute to enhanced cell growth rate. The cells with knockdown of MAP using target shRNA resulted in reduced hepatic mass forming and metastatic tumor nodules in orthotopic mice model. AKT activation by MAP linked to mTORC1 and GSK-3 β / β -catenin signalings, which are mainly associated with tumor cell growth and metastasis, respectively. mTORC1 and GSK-3 β / β -catenin signaling also appeared to affect each other. Epithelial-mesenchymal transition (EMT) was modulated by MAP/AKT and its downstream signaling in HCC. Through screening of expression profiles from a miRNA microarray and experimental analysis, we found that the miR-15b expression is down-regulated in overexpression of MAP in HLK3 cells, and increased in knockdown of MAP in Huh7 cells.

Conclusion: MAP may be involved in HCC growth and metastasis through AKT/mTORC1 and AKT/ β -catenin signaling pathways, which may be useful therapeutic targets.

O-150

Sharpin cooperates with Wnt pathway to transactivate Versican expression and promote HCC invasion

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Background: Sharpin (Shank-associated RH domain-interacting protein, also known as SIPL1) is a multi-functional molecule in various biological settings, including NF- κ B activation, inhibition of tumor suppressor gene and integrin signaling. Sharpin has been also reported to be overexpressed in hepatocellular carcinoma (HCC) and implicated in tumor progression; however its exact roles in tumor progression remain poorly understood. Here we report the novel mechanisms of HCC progression through Sharpin overexpression.

Methods: Stably Sharpin-expressing Huh7 cells were established, and its invasive properties were examined by matrigel invasion assay. The expression profiles of Sharpin-expressing cells were compared to control cells by cDNA microarray.

Results: Increased expression of Sharpin enhanced the invasion of hepatoma cells, whereas decreased Sharpin expression by RNA interference attenuated the invasion. cDNA Microarray analysis revealed upregulation of Versican, a component of the extracellular matrix chondroitin sulfate proteoglycans, in stably Sharpin-expressing cells. Knocking down of Versican greatly inhibited the invasion of hepatoma cells. Sharpin expression resulted in significant induction of Versican transcription synergistically with Wnt pathway activation. Furthermore, Sharpin binds to β -catenin, suggesting Sharpin might work as a coactivator of β -catenin.

Conclusions: These results demonstrated that Sharpin cooperates with Wnt pathway to transactivate Versican expression, which potentially contributes to HCC progression. Blockade of Sharpin/Versican axis could be an attractive therapeutic target in invasive HCC.

O-151

High NEK2 expression in hepatocellular carcinoma patients after hepatectomy

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Background: Better prognosis of caners including hepatocellular carcinoma (HCC) remains unsatisfactory due to recurrence and chemoresistance. Therefore, it is important to identify molecular targets involved in to design effective therapeutic strategies. In this study, we investigated the prognostic role of NIMA-related kinase 2 (NEK2) in HCC.

Methods: A total of 50 HCC patients who underwent hepatectomy were enrolled in this study. NEK2 gene and protein expressions were examined by quantitative real-time polymerase chain reaction (qRT-PCR) and Immunohistochemistry respectively. After HepG2 cells sphere formation, gene expressions in the parental and sphere formed cells were analyzed by qRT-PCR.

Results: High expression of NEK2 was detected in HCC tumor than adjacent non-tumor tissues ($P = 0.0001$), and the protein expression was also relatively high in tumor tissue than the corresponding non-tumor tissue. Furthermore, high NEK2 expression was positively correlated with hepatic venous invasion ($P = 0.047$), des-gam-macarboxy prothrombin ($P = 0.003$), and alpha-fetoprotein (AFP) ($P = 0.024$). Patients with high NEK2 expression had significantly poor recurrence-free survival ($P = 0.042$) and recurrence within 2 years ($P = 0.004$). Cancer spheres grown in serum-free non-adherent culture showed increased expression of NEK2 compared to parental cells grown in conventional culture.

Conclusions: NEK2 high expression may be a predictor of early recurrence and worse prognosis in patients with HCC.

O-152

NFATc3 exerts anti-viral and anti-tumor dual function by activating interferon pathwayXiangmei Chen¹, Jin Cheng², Zhenzhen Zeng¹, Fengmin Lu¹

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Background: Nuclear factor of activated T-cells, cytoplasmic 3 (NFATc3) can orchestrate an effective immune response in T cells. This study aimed to explore the function of NFATc3 in HBV-related hepatocellular carcinoma (HCC).

Methods: The function of NFATc3 in transcriptional regulation was detected by luciferase, real-time PCR and ChIP-PCR assays. The anti-HBV function of NFATc3 was evaluated in vitro and in vivo. The anti-tumor activity of NFATc3 was assessed in cell-based transfection assay.

Results: NFATc3 was down-regulated in HCC tissues compared to non-tumor tissues, and a decrease of NFATc3 was correlated with poor prognosis of HCC patients. Expression of NFATc3 in liver tissues was positively associated with the expression of IFNs, but was negatively associated with HBV viral load in HCC patients. Ectopic expression of NFATc3 significantly decreased the production of HBsAg, HBeAg, HBV RNA and total DNA in Hep3B cells transfected with 1.2x HBV replicon and HepAD38 cells stably producing HBV. Mechanistically, NFATc3 overexpression increased the transcriptional activity of both type I and II IFN genes which in turn upregulated the expression of interferon stimulated genes. IFN receptor blocker abolished the inhibitory effect of NFATc3 on HBV replication. Consistently, NFATc3 significantly decreased HBsAg and HBeAg level in peripheral blood in mouse model established by hydrodynamic tail vein injection. Lastly, restoration of NFATc3 significantly reduced the proliferation and migration of HCC cells.

Conclusion: NFATc3 exerts anti-viral and anti-tumor function by activating interferon pathway, which provides a novel option of clinical treatment for chronic hepatitis B and HBV-related HCC by targeting NFATc3.

O-153

Increased galectin-3 expression is related to poor prognosis in hepatocellular carcinomaFei Kong¹, Jing Jiang², Meishan Jin³

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Galectins (Gal) are multifunctional galectins that affect diverse physiological and pathophysiological processes such as development, inflammation, and tumor growth. A series of experimental have been reported to support a correlation between galectin expressions and neoplastic transformation, progression and prognosis. Our study was to evaluate the role of the expression of Gal-3 and Gal-9 during HCC occurrence and their prognostic values. Gal-3 and Gal-9 expression were evaluated using a tissue microarray immunohistochemistry method in 247 patients with HCC, of which 110 had paired adjacent

normal samples. Correlations were analyzed between expression levels of Gal-3 and Gal-9 protein and tumor parameters or clinical outcomes. Gal-3 expression in HCC was significantly higher than that in adjacent hepatic tissues (47.3 vs 18.2 %, $P = 0.001$), while no significant differences was observed in expression of Gal-9 ($P = 0.430$). High Gal-3 expression was statistically correlated with poor histological differentiation ($P = 0.016$), lymph-vascular invasion ($P = 0.049$) and no cirrhosis ($P = 0.040$). In contrast, low Gal-9 expression was related to poor differentiation ($P = 0.002$) and lymph-vascular invasion ($P = 0.012$). Kaplan-Meier analysis showed that patients with higher Gal-3 expression had worse overall survival ($P = 0.007$), however no relationship was found in Gal-9 expression and survival ($P = 0.146$). Multivariate analysis showed that multiple tumor (RR = 2.29, 95 % CI = 1.52–3.45), tumor size larger than 5 cm (RR = 1.72, 95 % CI = 1.14–2.60), Lymph-vascular invasion (RR = 1.68, 95 % CI = 1.07–2.64) and Gal-3 expression (RR = 1.83, 95 % CI = 1.16–2.87) were independent influencing factors of prognosis in patients with hepatocellular carcinoma. Gal-3 expression was involved in tumor progression and related to the prognosis of HCC, while Gal-9 expression was only related to tumor progression.

O-154

Molecular pathogenesis for HCC development: HSF1 potential molecular target for HCC therapiesMakoto Chuma¹, Naoya Sakamoto², Akira Nakai⁴, Hideki Yokoo³, Toshiya Kamiyama³, Akinobu Taketomi³, Kosuke Tashiro⁵, Kazushi Numata¹, Katsuaki Tanaka¹, Shin Maeda¹

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Hepatocarcinogenesis is closely associated with a stressful environment in the majority of cases. Heat shock factor 1 (HSF1), a major transactivator of stress responses, has been implicated in carcinogenesis in various organs. The aim of this study was to clarify the functional role of HSF1 in the development of HCC and cancer-related pathways regulated by HSF1, and also whether HSF1 and its regulated genes could be used as prognostic factors for HCC patients. We investigated deregulated pathways according to gain or loss of function of HSF1 in the Ingenuity Pathway Analysis (IPA) database. HSF1 regulated several molecules involved in many facets of tumorigenesis, including apoptosis, proliferation, energy metabolism, and invasion. Among these genes, we focused on genes associated with apoptosis and proliferation. Reduced tumorigenesis in HSF1-KD cells appeared attributable to increased apoptosis and decreased proliferation. Tumor necrosis factor alpha-induced apoptosis was increased in HSF1^{-/-} mouse hepatocytes compared with controls. Decreased expression of bcl-2-associated athanogene domain 3 (BAG3) was also observed in HSF1^{-/-} mouse hepatocytes, and might have been associated with increased apoptosis. We also found that epidermal growth factor mediated extracellular signal-regulated kinase (ERK) activation (P-ERK) was impaired in HSF1^{-/-} mouse hepatocytes. Clinicopathological analysis demonstrated frequent overexpression of HSF1, BAG3, and P-ERK in human HCCs. Significant correlations between HSF1 and BAG3 protein levels; HSF1 and P-ERK protein levels; and aggressive tumor factors and prognosis were also observed. These results suggest a mechanistic link between HSF1 and liver tumorigenesis

and may provide potential molecular targets for the development of anti-HCC therapies.

O-155

Hypoxia-activated cytotoxic tirapazamine kills HCC by hepatic artery ligation in HBx transgenic mice

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Transarterial chemoembolization (TACE) is the main treatment for intermediate stage of hepatocellular carcinoma (HCC), because of its exclusive arterial blood supply. However, TACE achieves only about 20–30 % of complete tumor remission rate and residual tumor generally recurs within a short period of time. To enhance the efficacy of TACE, we combined tirapazamine (TPZ), a hypoxia-activated cytotoxic agent, with hepatic artery ligation (HAL), which recapitulates transarterial embolization in mouse models. The therapeutic efficacy of this combinatorial treatment was examined in HCC spontaneously developed in HBx transgenic mice. We proved that the tumor blood flow in this model is exclusively supplied by hepatic artery, which is different from that in conventional orthotopic HCC xenografts receiving both the arterial and venous blood supplies. Below threshold oxygen by HAL, TPZ was activated and kill the hypoxic cells, but spare the normoxic cells. This combined treatment clearly limited the toxicity of TPZ only to HCC, which caused quick and near complete necrosis in HCC. In conclusion, combination of TPZ and HAL show a synergistic tumor killing activity specific for HCC in HBx transgenic mice. This preclinical work forms the basis for the ongoing clinical trial for the novel TPZ-TACE regimen in HCC treatment.

O-156

The clinicopathological analysis of collagen XV, which contributes sinusoidal capillarization

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Background: We have reported the specific expression of collagen XV in the human hepatocellular carcinoma (HCC), and shown to localize along the sinusoid-like vessels. The aim of this study was to explore the correlation between histopathological and molecular biological findings in HCC specimen and clinicopathological factors. **Methods:** Sixty-three HCC specimens were examined. Immunostaining of collagen XV and quantitative reverse transcriptional PCR of *COL15A1* were performed.

Results: Positive staining of collagen XV was observed in all tumoral regions regardless of differentiated level or pathological type of HCC; along with the sinusoid-like endothelium, though collagen XV was

not expressed in any non-tumoral region. The intensity of collagen XV-immunostaining and mRNA value of *COL15A1* were significantly correlated. The *COL15A1* expression in tumor elevated a 3.24-fold compared with non-tumoral regions and significantly correlated with non-viral and moderately differentiated HCC.

Conclusions: *COL15A1* mRNA was up-regulated in non-viral and moderately differentiated HCC. Our result suggest that the collagen XV expression contributes to the capillarization of all HCC, and the degree of contribution to capillarization is different.

O-157

ALBI grade vs. Child-Pugh grade as a grading system for liver function in patients with HCC

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Background and aim: The Albumin-Bilirubin (ALBI) grade has been proposed as a simple, objective method of assessing liver function. The prognostic performance of the ALBI grade in the patients with hepatocellular carcinoma (HCC) was investigated in this study.

Patients and methods: Total 2099 patients with HCC of the three tertiary hospitals in Korea were analyzed retrospectively. The discriminative performance in survival analysis of the ALBI grade was compared with the Child-Pugh(C-P) grade in different stages or treatments.

Results: The median follow up duration was 16.2 months (range 0–138.5). The median survival times were 49.7 months in C-P grade A (65.8 %), 12.4 months in C-P grade B (25.5 %), 4.2 months in C-P grade C (8.6 %) ($p < 0.001$, Harrell's C 0.68, Somers' D 0.36). The median survival times were 84.2 months in ALBI grade 1 (32.8 %), 25.5 months in ALBI grade 2 (53.5 %), 7.7 months in ALBI grade 3 (13.7 %) ($p < 0.001$, Harrell's C 0.682, Somers' D 0.364). In early UICC stages, the ALBI grade showed better discriminative performance than the C-P grade [Harrell's C: 0.676 (C-P grade) vs. 0.703 (ALBI grade), Somers' D: 0.353 (C-P grade) vs. 0.406 (ALBI grade)]. In curative treatments, the ALBI grade showed better discriminative performance than the C-P grade [Harrell's C: 0.624 (C-P grade) vs. 0.667 (ALBI grade), Somers' D: 0.248 (C-P grade) vs. 0.334 (ALBI grade)].

Conclusion: The ALBI grade had comparable discriminative performance in survival analysis and better distribution of the grades in HCC. It could be a good alternative grading system for liver function in patients with HCC.

O-158

Functional age is a stronger predictor of the outcomes of HCC than chronological age

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Background: There is a great diversity in the ability to tolerate treatments among elderly individuals. We hypothesized that functional age is a better predictor for survival of hepatocellular carcinoma (HCC) than chronologic age.

Methods: We reanalyzed a previously reported cohort of 1257 HCC patients (J Hepatol 2015), in which we measured skeletal muscle index (SMI) via CT scan. We evaluated whether functional age represented by SMI is a better prognosis indicator for HCC using multivariate Cox regression analysis.

Results: The cut-off values for SMI related to poor survival were 36.2 cm²/m² in males and 29.6 cm²/m² in females, respectively. We termed low SMI as sarcopenia. The advanced age defined as 75 or more years was a significant factor for all-cause mortality but not for liver-related mortality. In contrast, sarcopenia was significantly associated with both all-cause and liver related mortality. In multivariate analysis, sarcopenia (hazard ratio [HR], 1.54; 95 % confidence interval [CI], 1.20–1.99; $p < 0.001$) was a stronger factor associated with poor prognosis than advanced age (HR, 1.32; 95 % CI, 1.10–1.58; $p = 0.003$) adjusted by other significant factors. Interestingly, younger patients with sarcopenia showed poorer prognoses than elderly patients without sarcopenia (5-year survival rates, 35.3 versus 49.6 %).

Conclusion: Functional age represented by skeletal muscle mass is a stronger predictor of the outcomes of HCC than chronologic age. It is worth confirming whether strategy to increase skeletal muscle, such as exercise therapy and nutrition therapy, can improve the survival of HCC patients.

O-159

Clinical pattern of HCC in a single hospital: a report of 226 cases

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Background: Hepatitis viral infection is an important etiology of hepatocellular carcinoma (HCC). However, non-viral HCC is increasing which warrant further studies.

Objective: To compare demographic profiles and clinical characteristics between viral- and non-viral associated HCC patients.

Methods: A cross-sectional study was performed on HCC patients' database in Medistra Hospital from 2007-2015. Diagnosis of HCC was established by imaging modalities and alpha-feto protein (AFP). Demography and clinical data were obtained and analyzed. Categorical data were tested using Chi-square test, while continuous data were analyzed using the t test.

Results: There were 226 HCC cases retrieved from medical record data; etiologies were unknown in 14 cases and were excluded. Another two cases of autoimmune hepatitis were also excluded, leaving 210 cases available for further analyses. The most etiology of HCC is hepatitis B viral (HBV) infection (45.2 %) followed by non-viral etiology (28.1 %). Male patients were almost similar between viral and non-viral HCC (74.8 vs. 71.2 %; $p = 0.589$). Mean age of non-viral HCC patients was significantly older than viral HCC patients (67.4 + 11.39 vs. 61.2 + 12.59 years; $p = 0.001$). The presence of diabetes mellitus was significantly higher in non-viral HCC compared to viral HCC (50.8 vs. 28.5 %; $p = 0.002$). The presence of

liver cirrhosis were significantly higher in viral compared to non-viral HCC (80.0 vs. 65.5 %; $p = 0.03$).

Conclusion: Non-viral etiology of HCC found in almost one-third of all patients which could indicate the increasing metabolic factor as HCC risk factor. Compared to viral-associated HCC, the patients are older and are likely to have diabetes.

O-160

Access to healthcare did not change the clinical features and long-term outcomes of HCC in Japan

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Aim: To identify the change of clinical features at diagnosis and long-term outcomes of HCC, focusing on access to healthcare in a suburban area in Japan.

Methods: Collected data for 377 HCC patients (male 78 %, mean age 64.4 ± 9.4, 21 % residing on island) was analyzed. The era was divided into 2 groups: former era (before Apr 1999 when the bridge opened) and latter era.

Results: Distribution of Child-Pugh scores was as follows: A, 67 %; B, 20 %; and C, 13 %. HCC clinical stage was as follows: I, 14 %; II, 35 %; III, 31 %; IVa, 18 %; and IVb, 1.4 %. Patients in the latter era were diagnosed at earlier stages than that in the former era ($P < 0.05$). The latter era had a significantly longer survival rate than that of the former era (median observation period 814 days, 1-, 3- and 5-year survival: former era, 65, 32 and 19 %; and latter era, 84, 62 and 47 %, $P < 0.0001$). Child-Pugh score, HCC clinical stage and treatment were derived as a factor for survival (all $P < 0.0001$). However, there was no difference in terms of diagnosis at earlier stages and long-term outcomes between mainland and island. Multivariate analysis identified the era, Child-Pugh score, HCC clinical stage, and treatment as an independent factor for long-term outcome (all $P < 0.01$).

Conclusion: Long-term outcome in HCC patients has improved in a suburban area of Japan. However, this was not likely attributed to the change in access to healthcare but rather to an adherence to screening and surveillance programs and change in treatment strategy.

O-161

Urinary proton NMR spectroscopy in a Bangladeshi cohort as a fingerprint for HCC

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Aim: Urinary metabolic profiling using nuclear magnetic resonance (NMR) spectroscopy has an increasing role in providing a chemical fingerprint of hepatocellular carcinoma (HCC). Clinical HCC cohorts from Egypt and West Africa have been studied and this study aimed to establish if a similar urinary NMR profile could be identified in a Bangladeshi HCC cohort with different genetics, diet and environment.

Methods: Urine samples were collected, with informed consent, from patients attending the Bangabandhu Sheikh Mujib Medical University in Dhaka, from 42 patients with HCC, 47 with cirrhosis, 46 with chronic hepatitis B and 7 healthy controls. Urinary NMR data were acquired using a 600 MHz NMR spectrometer in London. The NMR data were initially analysed using principal component analysis (PCA) and then by univariate analysis for the most important discriminatory metabolites.

Results: Urinary NMR profiles from HCC differed to controls, non-cirrhosis and cirrhosis using PCA across range of metabolites, including acetate, creatine, creatinine, dimethylamine, formate, glycine, hippurate and trimethylamine-N-oxide. An independent-samples Kruskal-Wallis test showed a significant reduction in creatinine but increased creatinine levels in the HCC cohort compared to cirrhosis, and a reduction in hippurate levels in HCC compared to controls.

Conclusions: The urinary NMR changes in a Bangladeshi HCC population corroborate previous findings from African populations. These findings are consistent with the diverse effects of liver cancer on human physiology and gut bacterial action and may aid the development of a cost-effective HCC urinary dipstick screening test.

O-162

Transient elastography is useful for detecting high-risk patients of liver cancer

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Background and Aim: Liver cancers in patients without hepatitis B (HBV) and -C virus (HCV) infection are increasing in Japan. Method for detecting high-risk patients out of general population is still not established. Liver stiffness measurement (LSM) and Controlled Attenuation Parameter (CAP) using transient elastography (TE, Fibroscan®) are useful to detect liver fibrosis and steatosis. Aim of this study is to clarify the usefulness of LSM and CAP for selecting high-risk group of liver cancer out of general population.

Methods: This cross-sectional study was performed for residents aged >40 years in a town with a population of 3600. Blood laboratory testing, abdominal ultrasound (AUS), and TE were performed.

Results: Among 181 subjects (65 men, 116 women), LSM and CAP were measured in 175 subjects. The LSM was 7–12.5 kPa in 11 (6 %) and >12.5 kPa in 6 (3 %). Thirty nine percent of residents with a >7 kPa had HCV infection. We had set the CAP cutoff value for detection of steatosis by AUS as 242 dB/m, and defined high-risk group for liver cancer as >12.5 kPa or >7 kPa with CAP >242 dB/m. Values for BMI, AST, ALT, GGT, and AFP were significantly higher in the high-risk group compared with the other subjects. Ultimately, 3 patients of hepatocellular carcinoma (HCC) were detected.

Conclusion: In the present study, 3 patients of HCC were detected. High risk group which were defined by LSM and CAP also included the HCC patient. TE can potentially identify high-risk group.

O-163

Intraoperative detection of superficial liver tumors by fluorescence imaging using ICG and 5-ALA

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Background: Indocyanine green (ICG) and the porphyrin precursor 5-aminolevulinic acid (5-ALA) have been approved as fluorescence imaging agents in the clinical setting. This study evaluated the usefulness of fluorescence imaging with both ICG and 5-ALA for intraoperative identification of latent small liver tumors.

Methods: There were 48 patients who had main tumors within 5 mm of the liver surface. 5-ALA hydrochloride was orally administered to patients 3 h before surgery. ICG had been intravenously injected within 14 days prior to surgery. Intraoperatively, after visual inspection, manual palpation, and ultrasonography, fluorescence images of the liver surface were obtained with ICG and 5-ALA prior to resection.

Results: With ICG, the sensitivity, specificity, and accuracy for detecting the preoperatively identified main tumors were 96, 50, and 94 %, respectively. Twelve latent small tumors were newly detected on the liver surface using ICG, five of which proved to be carcinomas. With 5-ALA, the sensitivity, specificity, and accuracy for detecting the main tumors were 57, 100, and 58 %, respectively. Five latent small tumors were newly detected using 5-ALA; all were carcinomas. Overall, five new tumors were detected by both ICG and 5-ALA fluorescence imaging; two were hepatocellular carcinomas and three were metastases of colorectal cancer. The sensitivity and specificity of ICG fluorescence imaging for main tumor detection were relatively high and low, respectively, but the opposite was true of 5-ALA imaging.

Conclusions: Fluorescence imaging using 5-ALA may provide greater specificity in the detection of surface-invisible malignant liver tumors than using ICG fluorescence imaging alone.

O-164

New Fusion Technology; Colorezid RVS

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Background: In this study, we evaluated the usefulness of a novel real-time virtual sonography (RVS) method that collates multiple DICOM data sources and displays reference images in color.

Methods: Using the SYNAPSE VINCENT volume analyzer, DICOM data of the hepatic segments were isolated from contrast-enhanced computed tomography DICOM data. Each portion of DICOM data was uploaded into an US scanner (HI VISION Ascendus, Hitachi Aloka Medical Ltd, Tokyo, Japan) and unified on a US platform to create a single reference image. Each uploaded portion of DICOM data was assigned a different color. Further, conventional RVS was performed using this information.

Results: We evaluated 7 patients with 9 hepatocellular carcinomas. The maximal tumoral diameter ranged from 6.4 to 15 mm

(mean \pm SD, 11.0 \pm 2.8). DICOM data could be isolated, enabling the display of color RVS in all patients. Color RVS facilitated superior visibility compared with conventional grayscale RVS and facilitated the comprehension of spatial positioning.

Conclusion: RVS with color display has utility in increasing operator comprehension of spatial and positional relationships during percutaneous US examination.

O-165

Risk factor of distant recurrence of HCC following RFA; Gd-EOB-DTPA-enhanced MRI based analysis

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Background: The gadolinium-ethoxybenzyl-diethylenetriamine pentaacetic acid (Gd-EOB-DTPA)-enhanced magnetic resonance imaging (MRI) performed before curative therapy for hepatocellular carcinoma (HCC) has utility in distinguishing between distant recurrence (intrahepatic metastasis and de novo recurrence) and hypervascularization. However, whether the presence of non-hypervascular hypointense nodules is a risk factor of distant recurrence after curative therapy is yet controversial. Therefore, we aimed to retrospectively evaluate the presence of non-hypervascular hypointense nodules on hepatobiliary phase images (HBPI) on Gd-EOB-DTPA-enhanced MRI as a risk factor of distant recurrence of early-stage HCC following radiofrequency ablation (RFA).

Methods: A total of 132 patients who underwent pre-procedural Gd-EOB-DTPA-enhanced MRI followed by initial RFA were retrospectively analyzed. Post-RFA distant recurrence, which excluded hypervascularization of non-hypervascular hypointense nodules detected by pre-procedural Gd-EOB-DTPA-enhanced MRI, was evaluated according to the presence of non-hypervascular hypointense nodules on pre-procedural Gd-EOB-DTPA-enhanced MRI.

Results: Distant recurrence rates following RFA were higher in patients with non-hypervascular hypointense nodules. The presence of non-hypervascular hypointense nodules was an independent risk factor of post-RFA distant recurrence [hazard ratio (HR) 2.763]. The presence of non-hypervascular hypointense nodules was associated with significantly increased cumulative recurrence rates of both intra- and extra-segment distant recurrence and was demonstrated as an independent risk factor of post-RFA intra-segment (HR: 2.772) and extra-segment distant recurrence (HR 3.175).

Conclusion: The presence of non-hypervascular hypointense nodules on HBPI from Gd-EOB-DTPA-enhanced MRI obtained prior to RFA is a significant predictive factor of distant recurrence following RFA of early-stage HCC.

O-166

Locoregional therapy or liver transplantation for hepatocellular carcinoma fulfilling Milan criteria

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Patients with hepatocellular carcinoma (HCC) satisfying the Milan criteria is candidates for liver transplantation (LT), but also locoregional therapies can be considered. There is uncertainty which treatment is first-line option for these patients. A total of 1859 treatment-naïve, HCC patients fulfilling the Milan criteria were analyzed. Survival tree analysis was done to generate survival nodes with similar survival, and was compared to 130 patients received LT during follow-up. The 5-year survival for patients received LT was 81.5 %. Among patients who did not received LT, survival-tree analysis classified patients into six nodes according to the Child-Pugh (CP) score, serum alphafetoprotein (AFP) levels, tumor size and age, with different risk for survival (5-year survival of 87.3, 77.5, 65.8, 64.7, 44.0 and 28.7 % for node 1, 2, 3, 4, 5 and 6, $p < 0.001$). The overall survival of node 1 (non-LT) and 2 was comparable to LT ($p > 0.05$ for node 1, 2), but survival of node 3, 4, 5 and 6 was worse than LT ($p < 0.05$ for all). Node 1 was characterized by CP score 5 with low serum AFP levels (< 5 ng/ml), and node 2 was characterized by CP score 5 and maximal tumor size < 2.5 cm. Among patients who were diagnosed within the Milan criteria, similar survival can be expected by locoregional therapy for patients with preserved liver function and low AFP or preserved liver function and small tumor size.

O-167

Prognosis after resection of locally recurrent HCCs following four types of local treatment

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Background: Several studies showed that locally recurrent hepatocellular carcinomas (LR-HCCs) after radiofrequency ablation (RFA) were more invasive.

Methods: The patients who underwent hepatectomy for LR-HCCs between 1998 and 2014 were reviewed. Tumor profiles and patients' prognosis were compared among the different types of preceding local treatment.

Results: Among 901 hepatectomy, LR-HCCs that appeared after hepatectomy (Hx), RFA, percutaneous ethanol injection (PEI), and transarterial chemoembolization (TACE) were resected in 16, 31, 16, and 65 patients, respectively. Among the four groups, preoperative ICG-R15, α -fetoprotein and des-gamma carboxyprothrombin, tumor size, and number, were comparable, while tumor size at the time of previous treatment (median [cm]; Hx vs. RFA vs. PEI vs. TACE, 4.5 vs. 2.0 vs. 2.4 vs. 3.0, $P < 0.01$) was different. The incidence of microscopic (44–52 %) and macroscopic vascular invasion (13–20 %) were comparable, while poorly differentiated or undifferentiated HCCs were less frequent in the RFA group (19 vs. 10 % vs. 44 vs. 28 %; $P = 0.06$). Postoperative median recurrence free survival of the whole 128 patients was 15 months, but 82 of 98 patients with recurrence underwent further local treatment, and

median overall survival was 67 months. Independent prognostic predictors were tumor size ≥ 5 cm at the time of previous treatment, multiple tumors at the time of previous treatment, and α -fetoprotein ≥ 400 ng/ml. Postoperative survival was comparable among the four groups ($P = 0.92$).

Conclusion: LR-HCCs after RFA are not associated with more invasive pathologic profiles. Hepatectomy for LR-HCCs can be helpful as a bridge to further local treatment, which leads to long survival irrespective of the types of preceding treatment.

O-168

Multiple positive tumor markers predict poor survival in patients with hepatocellular carcinoma

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Background: Several studies have evaluated the relevance of the expression pattern of the three tumor markers alpha fetoprotein (AFP), the isoform AFP-L3, and des-gamma-carboxy prothrombin (DCP) with regard to prognosis in patients with hepatocellular carcinoma (HCC). We retrospectively evaluated the influence of the expression pattern of these three tumor markers on the prognosis of patients with HCC within the Milan criteria and good liver function. **Methods:** From January 1994 to December 2014, 1182 consecutive patients underwent hepatic resection and surgical microwave ablation for HCC at our institute. Of these, we analyzed 475 patients within the Milan criteria and Child-Pugh class A. Cumulative disease-free survival and overall survival were analyzed according to the number of positive tumor markers.

Results: Overall survival and disease-free survival at 5 years postoperatively were 85.3 and 44.2 % in all tumor marker-negative patients, 79.4 and 48.0 % in single-positive patients, 56.2 and 32.9 % in double-positive patients, and 61.7 and 35.7 % in triple-positive patients with statistical significance. Overall survival in all negative or single-positive patients was 85.3 %, and that in all double- or triple-positive patients was 58.0 % ($P < 0.0001$); disease-free survival at 5 years postoperatively in these two groups was 45.9 and 34.0 %, respectively ($P = 0.0013$). Multivariate analyses revealed double- and triple-positive tumor markers to be independent risk factors for early recurrence and poor survival.

Conclusions: Both double- and triple-positive tumor markers are associated with early recurrence and poor survival in patients with HCC within the Milan criteria and Child-Pugh class A.

O-169

Comparison of HAIC and sorafenib in HCC patients refractory to TACE

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Aim: To compare efficacy of hepatic arterial infusion chemotherapy (HAIC) and sorafenib in hepatocellular carcinoma (HCC) patients refractory to transcatheter arterial chemoembolization (TACE).

Methods: 59 HCC patients refractory to TACE with Child-Pugh A, no extrahepatic metastasis and no macroscopic vascular invasion were enrolled in this study. Using one to one case control method, overall survival (OS) and time to progression (TTP) were compared between HAIC group ($n = 20$) and sorafenib group ($n = 20$).

Results: There were no significant differences between these groups about clinical backgrounds (age, gender, liver functional reserve, main tumor size and value of tumor marker). The median survival time was better in sorafenib groups (16 M) than in HAIC group (7 M) ($p = 0.006$). TTP was also better in sorafenib group than in HAIC group.

Conclusions: Sorafenib showed favorable OS and TTP among HCC patients refractory to TACE compared with HAIC.

O-170

Survival Outcomes of Cytoreductive Surgery for Advanced Hepatocellular Carcinoma

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Background: In the treatment of hepatocellular carcinoma (HCC), cytoreductive liver surgery is performed in selected cases with (1) oncological emergency such as tumor thrombus in the inferior vena cava (IVCTT) or tumor rupture (Group E), (2) unresectable small extra-hepatic metastatic foci and a large intra-hepatic tumor (Group M), and (3) multiple intra-hepatic tumors in whom complete resection of the tumors is difficult due to poor liver functional reserve (Group R). The aim of the present study was to assess the significance of these cytoreductive surgery focusing on the possibility of subsequent treatments and survival outcomes.

Methods: Our medical database from 2001 through 2015 was reviewed, and 34 HCC cases receiving cytoreductive surgery were identified. The contents of postoperative treatments and survival outcomes were investigated.

Results: The study cohort consisted of 5 Group E cases, 13 Group M cases, and 16 Group R cases. Postoperative treatments were performed in 4 Group E cases, 10 group M cases, and 13 group R cases. The treatment modality was TACE ($n = 17$), chemotherapy ($n = 6$), molecular-targeted therapy ($n = 4$) and radiation ($n = 4$). The median survival time was 5.7 months (range 2.2–23.5 months) in Group E, 5.3 months (range 1.4–11.5 months) in Group M, and 16.8 (1.7–68.4 months) in Group R, respectively.

Conclusions: Long survivors were rare in cases receiving cytoreductive surgery, but surgery combined with repeated TACE provided survival benefit in selected cases in Group R. In Group E cases, complete resection is mandatory to obtain survival benefit. Powerful chemotherapy regimens are required for Group M cases.

O-171

Factors relevant to incomplete ablation at first session of RFA in 403 HCC patients

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The aim of this study is to evaluate factors relevant to incomplete ablation at first session of radiofrequency ablation (RFA).

We performed RFA percutaneously on 403 patients with HCC at our institution between October 2014 and August 2015. When tumors were inconspicuous, we used the multimodality fusion imaging and contrast-enhanced ultrasonography to identify the tumors. 1–3 day after RFA, we performed computed tomography (CT) to assess whether targeted HCCs were ablated completely. When CT showed a possible residual tumor portion, we performed additional RFA until we got adequate ablation area.

Complete ablation was achieved in 365 patients (complete ablation group) and viable cancer tissue remained in 38 patients (incomplete ablation group) at first session. Technical success rate at first session and final session was 90.5 % (365/403) and 99.5 % (401/403), respectively. The differences in patient characteristics and treatment factors between the two groups were not statistically significant except for number of tumor ($P < 0.001$), maximum tumor diameter ($P = 0.03$), within Milan criteria ($P < 0.001$), number of puncture ($P = 0.03$) and total ablation time ($P = 0.03$). In multivariate logistic regression analysis, number of tumor (odds ratio 0.69; 95 % confidence interval 0.58–0.82; $P < 0.001$) and maximum tumor diameter (odds ratio 0.97; 95 % confidence interval 0.94–1.00; $P = 0.046$) were the significant factors relevant to incomplete ablation at first session of RFA.

This results demonstrated that number of tumor and maximum tumor diameter were the risk factors relevant to residual tumor portion at first session in RFA for HCC.

O-172

Postoperative Outcomes for Patients with HBcAb-positive Non-B Non-C Hepatocellular Carcinoma

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Backgrounds/Aims: So-called non-B non-C hepatocellular carcinoma (nBnC-HCC), which is not associated with active hepatitis B or hepatitis C virus infection, has been regarded to be correlated with better survival compared to HBV- or HCV-related HCC. However, nBnC-HCC includes with HCC related to past history of HBV infection possessing anti-hepatitis B core antibodies (HBcAb). The oncological significance of past history of HBV infection remains unclear.

Methods: The records of 562 patients undergoing curative resection for primary HCC were retrospectively reviewed. The long-term outcomes were compared among the 4 groups stratified by viral infection status: HBV group (HBsAg-positive), HCV group (HCVAb-positive), HBcAb-positive nBnC-HCC group, and pure nBnC-HCC group (negative for these viral markers).

Results: The HBcAb-positive nBnC-HCC group showed better overall survival (OS) rate (5-year OS, 89.4 vs. 68.4 %, 62.0, and 66.2 %; $P = 0.003$) and recurrence-free survival (RFS) rate (5-year RFS, 53.8 vs. 31.4 %, 28.1, and 33.6 %; $P = 0.01$), compared with HBV, HCV, or pure nBnC-HCC groups, respectively. According to the multivariate analysis, the past history of HBV infection was associated with a lower risk of OS [Hazard ratio (HR), 0.23; 95 % CI 0.09–0.56; $P = 0.001$] and RFS (HR, 0.45; 95 % CI 0.27–0.73, $P = 0.001$). When comparing with the pure nBnC-HCC group, the HBcAb-positive nBnC-HCC group showed higher incidence of well

differentiated HCC (33 vs. 15 %, $P = 0.03$) and lower plasma des-gamma-carboxyprothrombin concentration (72 vs. 357 mAU/mL, $P = 0.047$).

Conclusions: The subgroup of patients with a history of HBV infection may have better survival outcomes after resection of HCC than the HBV/HCV-related or pure nBnC-HCC patients.

O-173

Efficacy and safety of sorafenib in patients with non-B, non-C hepatocellular carcinoma in Japan

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Aim: Sorafenib has been used as the standard treatment for advanced hepatocellular carcinoma (HCC), however, its efficacy and safety in patients (pts) who were negative for hepatitis B virus surface antigen and anti-hepatitis C virus antibody (non-B, non-C), have not yet been fully examined. This study was conducted to evaluate the efficacy and safety of sorafenib in pts with non-B, non-C HCC.

Method: A total of 83 non-B, non-C HCC pts treated with sorafenib from November 2007 to February 2015 were retrospectively reviewed. Tumor response, overall survival (OS), Time to progression (TTP), and toxicities were analyzed.

Results: There were 70 males and 13 females with a median age of 72 years. 63 patients had Child-Pugh class A cirrhosis and 20 patients had Child-Pugh class B cirrhosis and 46 pts had history of heavy alcoholic consumption and 40 pts received diabetes mellitus treatments. 6 pts (7 %) achieved partial response, and 37 pts (44 %) showed stable disease. The median OS and TTP was 8.3 and 2.5 months, respectively. With regard to toxicities, the main grade 3/4 toxicities were, hand foot skin reaction, thrombocytopenia, elevated aspartate aminotransferase and elevated alanin aminotransferase. There were no treatment-related deaths.

Conclusions: Sorafenib showed modest efficacy and manageable toxicity and in patients with non B non C hepatocellular carcinoma, consistent with other hepatitis virus related HCC reports. The results could be useful as reference data for optimizing treatment strategies and planning future clinical trials.

O-174

Comparative analysis of miriplatin and doxorubicin in TACE for HCC using propensity score matching

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Aim: The choice of the anticancer agent of conventional transcatheter arterial chemoembolization (cTACE) for hepatocellular carcinoma (HCC) is not uniformly. The aim of this study was to compare miriplatin (MPT) and doxorubicin (DXR) in cTACE for HCC using propensity score matching.

Methods: Between 2005 and 2014, we retrospectively collected data of 162 patients who underwent MPT or DXR in the first cTACE for HCC. Among them, 68 patients enrolled in this study and divided into

two groups, MPT or DXR in cTACE using propensity score one-to-one matching. We compared outcomes between the two groups.

Results: After propensity matching, objective response rate was 64.7 % in MPT and 79.4 % in DXR, however, treatment effect (TE)4 rate and continuation rate of TE4 was higher DXR than MPT. There were no significant differences between the two groups overall survival, time to progression. Multivariate analysis revealed CLIP score and albumin and CRP to be significant prognostic factors and identified tumor numbers and MPT in cTACE to be significant risk factors associated with no response to TE4.

Conclusion: DXR was higher TE4 rate and TE4 continuation rate than MPT. DXR is an effective treatment with regard to local control in cTACE for HCC.

O-175

Sequential therapy (PEI-RFA at first-IVR at second for large sized hepatocellular carcinoma)

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According to algorithm for the treatment of liver cancer larger than 3 cm in diameter, TAE or resection is first choice of the treatment. However, some cases exist difficult to treat. We've been reporting the effectiveness of the combination therapy of ethanol injection and RFA (PEI-RFA) for inducing large amounts of coagulated necrosis by small amounts of energy output. Thus, we performed combined sequential therapy PEI-RFA at first-IVR at second for the treatment of large sized HCC.

Methods: PEI-RFA was performed by injecting the ethanol into the tumor prior to RFA. IVR was performed by injecting miriplatin hydrate and 5FU.

Results: By the first PEI-RFA treatment, large amounts of HCC could be ablated at one time and almost the entire area of mass reduction could be achieved. By adding IVR treatment, it was possible to achieve CR in some cases with large sized HCC.

Case presentation: A 70 years old woman lived in Shodoshima, Japan admitted with HCC 5 cm in diameter located in the right lobe of the liver 7 years ago. Combined sequential therapy PEI-RFA-IVR was performed for the treatment. Even now 7 years after the treatment, she is healthy without causing recurrence of liver cancer.

Conclusions: Combined PEI-RFA-IVR is effective for the treatment of large sized HCC larger than 3 cm in diameter.

O-176

Post-progression survival in patients with advanced hepatocellular carcinoma resistant to sorafenib

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Background: After the approval of sorafenib, no other agent has proven survival benefits in clinical trials involving patients with

advanced hepatocellular carcinoma (HCC) resistant to sorafenib. Prognostic factors for survival after tumor progression in sorafenib-treated patients are critical for designing second-line trials.

Methods: A prospective study was conducted on patients with advanced HCC treated with sorafenib at Chiba University Hospital. To determine the factors affecting the post-progression survival (PPS) after sorafenib treatment, these patients were analyzed in view of patient characteristics at the time of tumor progression and progression pattern (intra-/extrahepatic growth or emergence of new intra-/extrahepatic lesions) were analyzed.

Results: Of 89 enrolled patients, 70 were diagnosed with disease progression according to the Response Evaluation Criteria in Solid Tumors version 1.1. PPS of all patients was 8.1 months (95 % CI 5.5–10.9). Multivariate Cox's regression analysis revealed that Child-Pugh scores 7 or more, macrovascular invasion (MVI), and alpha-fetoprotein of >400 ng/mL are independent predictors of poor PPS. Although both EHM and MVI are characteristics of advanced HCC, EHM was not determined as a prognostic factor. Additionally, emergence of new extrahepatic lesions also serves as an independent poor prognostic factor. The PPS of the patients was well stratified according to the index based on the sum of these prognostic factors, ranging 0–4.

Conclusions: These prognostic factors for PPS may be utilized for the prognosis prediction and taken into consideration while designing second-line trials.

O-177

A case of recurrent HCC with bile duct invasion effectively treated by cyberknife

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A 69 year-old cirrhotic male patient treated previously for HCC presented the dilatation of intrahepatic bile duct, B6, together with the recurrent HCC close to portal area on follow-up CT. As loco-regional treatment, we chose Cyberknife (CK), a stereotactic body radiotherapy, to avoid severe bile duct injury by RFA. Two months after the completion of CK, the elevated hepatobiliary enzyme test and the worsening of biliary dilatation was found. He was hospitalized for the investigation. ERCP showed the filling defect of the right hepatic duct, and tumor invasion was suspected by intraductal ultrasound (IDUS). A plastic bile duct stent was placed for biliary drainage. Biopsy specimen revealed bile duct invasion of HCC. After 3 months, the finding of duct invasion disappeared on both ERCP and IDUS, and the stent was removed. We thought CK affected the lesion effectively because no other treatment had been done. In Japan, CK has been approved by health insurance for neoplasms of body trunk, including hepatic cancer, since 2008. Previous reports showed that the effect of CK on HCC was relatively slow and might take 6 months for the disappearance of the viable tumor cells. In our case, it took 5 months to confirm response after the transient exacerbation evaluated by conventional imagings, e.g. CT and cholangiography. Despite of difficulties for the assessment of response, CK might be a promising treatment option for HCC with bile duct invasion hardly controlled by other modalities.

O-178

Whole liver chemoembolization with cisplatin and trisacryl gelatin microsphere for multiple hepatoma

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Purpose: The purpose of this study is to evaluate the safety and effectiveness of whole liver transcatheter arterial chemoembolization (TACE) with Cisplatin fine powder and trisacryl gelatin microsphere for the treatment of unresectable multinodular hepatocellular carcinoma (HCC).

Materials and methods: All patients with nonresectable multinodular HCC who underwent whole liver TACE sessions with Cisplatin fine powder (IA Call®; Nippon Kayaku, Tokyo, Japan) and trisacryl gelatin microsphere (Embosphere®, Nihon Kayaku, Tokyo, Japan) from September 2014 to June 2015 were retrospectively reviewed. The cisplatin dose was 65 mg/m². The cisplatin dissolved in 70 ml of saline. The cisplatin solution was infused through a microcatheter placed nonselectively in the proper hepatic artery for 20 min to allow for full exposure of all tumors to the drug. Nonselective TACE was performed until all tumor enhancements disappeared. Toxicity was assessed by Common Toxicity Criteria for Adverse Effects (version 3.0), and tumor response was evaluated by modified Response Evaluation Criteria in Solid Tumors (mRECIST).

Result: Eight patients (5 males, 3 females, mean age 69.1 years) were included in the analysis. A total of 10 procedures were performed. There were no severe adverse events except for transient grade 3 increase of AST and ALT in one patient. Objective Response Rate was 40.0 % and Disease Control Rate was 60.0 % by mRECIST.

Conclusions: Whole liver TACE with Cisplatin fine powder and trisacryl gelatin microsphere was well tolerated and effective in patients with unresectable multinodular HCC.

O-179

GALNT14 genotype correlates with cholangiocarcinoma features, chemoresponse and overall survival

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Background: Cholangiocarcinoma is a rare but aggressive cancer. Only patients diagnosed in early stages receive curative surgical treatments. Genetic factors associated with cholangiocarcinoma are elusive. Here, we investigated whether GALNT14-rs9679162 genotype, a genotype related to hepatoma chemotherapy responses, is also associated with the therapeutic outcomes in cholangiocarcinoma.

Methods: A cohort of 112 resected cholangiocarcinoma patients was retrospectively recruited. GALNT14 genotypes were correlated with clinical factors and postoperative prognosis.

Results: Of these patients, 31.3, 49.1 and 19.6 % had GALNT14 TT, TG and GG genotypes, respectively. Perineural invasion and lymph node involvement independently associated with the TT genotype ($P = 0.035$ and 0.005 , respectively). In a subset of patients ($n = 33$)

who received chemotherapy following tumor recurrence, the genotypes were associated with chemotherapy response ($P = 0.001$). Longitudinal data showed that the genotype TT was associated with unfavorable overall survival (log-rank $P = 0.023$, Cox $P = 0.027$). Multivariate analysis showed that perineural invasion and vascular invasion were independently associated with overall survival ($P = 0.001$ and 0.002 respectively).

Conclusions: GALNT14 genotypes were associated with perineural invasion, lymph node metastasis, chemotherapy response and post-operative overall survival in cholangiocarcinoma patients.

O-180

Impact of preoperative sarcopenia on outcomes after resection of biliary cancer.

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Background: Skeletal muscle depletion, referred to as sarcopenia, predicts mortality after surgery. This study investigated the impact of preoperative skeletal muscle quantity and quality on outcomes in patients undergoing resection of biliary cancer.

Methods: We performed a retrospective analysis of 207 patients undergoing resection for biliary cancer between 2004 and 2013. The quantity and quality of skeletal muscle, indicated by psoas muscle mass index (PMI) and intramuscular adipose tissue content (IMAC), were measured on preoperative computed tomography images. Overall survival (OS) and recurrence-free survival (RFS) rates were compared by PMI and IMAC, and prognostic factors after operation were assessed.

Results: The OS and RFS rates were significantly lower in patients with low PMI (low muscle quantity) than in those with normal PMI ($P < 0.001$, $P < 0.001$) (5-year OS: 15.7 vs. 53.5 %). The OS and RFS rates were also significantly lower in patients with high IMAC (low muscle quality) than in those with normal IMAC ($P < 0.001$, $P < 0.001$) (5-year OS: 23.8 vs. 55.9 %). Low PMI and high IMAC were independent factors predictive of poor OS [Hazard ratio (HR) = 2.921, 95 % confidence interval (CI) 1.920–4.470, $P < 0.001$; HR = 1.725, 95 % CI 1.159–2.590, $P = 0.007$] and RFS (HR = 2.141, 95 % CI 1.464–3.129, $P < 0.001$; HR = 1.492, 95 % CI 1.032–2.166, $P = 0.034$).

Conclusions: Preoperative sarcopenia, indicating low quantity and quality of skeletal muscle, is closely related to mortality after resection of biliary cancer.

O-181

Clinical significance of lymph node dissection for intrahepatic cholangiocarcinoma

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Background: Intrahepatic cholangiocarcinoma (ICC) is a lethal neoplasm originating from the biliary tree, and the incidence is increasing. While surgery resection of tumor lesion is the optimal approach to ICC, it is still controversial about adding the lymph node dissection (LND), in order to improve the dismal prognosis.

Patients and methods: A retrospective analysis was conducted on 78 ICC patients who had undergone surgical resection of ICC in our institute. We evaluated the effect of LND and investigated the surgical indication of LND from the information of those clinicopathological features and the prognosis. In this time, we divided ICC into two types according to the location; Hilum type, Main tumor location is within first branch from common bile duct; Peripheral type, Main tumor location is over first branch.

Results: LND were performed 46 cases (59 %), and 22 cases (28 %) showed LN metastasis in or after surgery. The multivariate following univariate analysis indicated only two significant factors associating poor prognosis; the high preoperative CA19-9 (>200), the type of tumor localization (Hilum type). In Hilum type ICC cases (n = 31), LND were performed in 31 cases and lymph node metastasis were detected in 14 cases (45 %). Nine of twenty patients with high preoperative CA19-9 were indicated lymph node metastasis by the preoperative image investigation and therefore performed LND. Three patients in the rest of eleven patients met lymph node metastasis after surgery.

Conclusion: Routine LND are not necessary for every peripheral ICC patients, but for patient with Hilar ICC or showing high preoperative CA19-9 (>200).

O-182

A novel prognosis scoring system for intrahepatic cholangiocarcinoma

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Intrahepatic Cholangiocarcinoma (ICC) is a poorly understood biliary malignancy, but the prognostic factors have not been well established. To improve prognostic scoring system, we screened the protein which up regulated in ICC tissues using relative and absolute quantitation (iTRAQ) proteomic technique. After, narrow down by using bioinformatics analysis, western blot, immunohistochemistry, and Cox multivariate analysis, a novel prognostic system was developed which including pyruvate kinase M2 (PKM2) and other three proteins and clinicopathologic characteristics for ICC patients after surgery. The prognostic scoring system was constructed in combined primary (328 ICC patients) and validation cohort (219 ICC patients) and was assed both in a primary cohort of and validation cohort. In a multivariate COX analysis, the novel prognostic scoring system preferably predicts overall survival (OS) and time to recurrence (TTR) in primary cohort and in an independent validation cohort. Furthermore, the area under curves also revealed that the novel prognostic scoring system superior to 7th edition of the American Joint Committee on cancer staging system in determining OS and TTR for ICC patients. Application of the novel prognostic scoring system will permit more selective use of therapies for ICC patients treated surgically, improved outcomes for such individuals, and leading to provide a unified framework for further clinical and preclinical translational research.

O-183

Primary sclerosing cholangitis complicated with cholangiocarcinoma

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Background: Primary sclerosing cholangitis (PSC) complicated with cholangiocarcinoma (CC) is rarely reported in Japan.

Methods: In this study, we investigated 38 patients with PSC retrospectively. A total of 5 patients (13.2 %) had CC. This rate corresponds to that reported in Western countries.

Results: The mean age at diagnosis of CC was 49.6 years (range 31–65 years). Two patients were women. Three of 5 patients did not suffer from cirrhosis. No significant difference was observed in background characteristics between patients with and without CC. Three of 5 patients were diagnosed with CC at the same time or within the first year after diagnosis of PSC. CC manifested as bile duct stricture or mass formation. Two of 5 patients exhibited mass formation. The prognosis of patients with CC was poor, and all patients died. The median survival time was 1.04 years.

Conclusions: Patients with PSC should be carefully monitored for CC development using an imaging modality, particularly within the first year after diagnosis of PSC.

O-184

Recurrence of intraductal papillary neoplasm of the intrahepatic bile duct: Report of two cases

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Aim: Intraductal papillary neoplasm (IPN) of the intrahepatic bile duct is an infrequent biliary neoplasia, and few studies have investigated about recurrence pattern of IPN. Aim of this study is to examine the clinicopathological features of recurred IPN using a clinicopathological approach.

Method: We analyzed the characteristics of two cases of recurred IPN of the intrahepatic bile duct.

Results: Case 1: A 65-year-old man was found to have a hilar mass lesion with bile duct dilatation on imagings and underwent left hepatectomy. The resected tumor was composed of a papillary arrangement of tumor cells with microinvasion. There was no mucin hypersecretion. Immunohistochemical analysis revealed MUC1 and MUC5AC expression. The tumor was diagnosed as pancreatobiliary type IPN with negative surgical margin, although a recurred IPN was found at the intrapancreatic bile duct after 2 years. Recurred IPN showed similar histological features to primary tumor. Case 2: A 78-year-old man had a mass lesion arising from the left hepatic duct. Left hepatectomy was performed for the tumor which histologically showed papillary or tubular structures without mucin hypersecretion.

No invasion was identified. Immunohistochemistry showed MUC1 expression only. The tumor was diagnosed as pancreatobiliary type IPN with negative surgical margin. However, two recurrent lesions appeared at extrahepatic bile duct after 3 years. Recurrent tumors showed well differentiated tubular adenocarcinoma.

Conclusion: The two IPN cases with negative surgical margin recurred after surgical resection, suggesting that margin status is a relatively poor predictor of local recurrence for these cases.

O-185

Zolpidem use associated with increased risk of pyogenic liver abscess

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Background: The purpose of this study was to explore the association between zolpidem use and pyogenic liver abscess in Taiwan.

Methods: This was a population-based case-control study using the database of the Taiwan National Health Insurance Program since 2000 to 2011. We identified 1325 subjects aged 20–84 years with the first-attack of pyogenic liver abscess as the cases and 5082 subjects without pyogenic liver abscess matched with sex, age, comorbidities and index year of hospitalization for pyogenic liver abscess as the controls. Subjects whose last remaining one tablet for zolpidem was noted <7 days before the date of admission for pyogenic liver abscess were defined as current use of zolpidem. Subjects whose last remaining one tablet for zolpidem was noted >7 days before the date of admission for pyogenic liver abscess were defined as late use of zolpidem. Subjects who never received 1 prescription for zolpidem were defined as never use of zolpidem.

Results: After adjustment for possible confounding variables, the adjusted odds ratio of pyogenic liver abscess was 3.89 for subjects with current use of zolpidem (95 % confidence interval 2.89, 5.23), when compared with those with never use of zolpidem. The adjusted odds ratio decreased to 0.85 for those with late use of zolpidem (95 % confidence interval 0.70, 1.03), but without statistical significance.

Conclusions: Current use of zolpidem is associated with increased risk of pyogenic liver abscess. Physicians should take the risk of pyogenic liver abscess into account when prescribing zolpidem.

O-186

Routine Gd-EOB-MRI is recommended for the preoperative evaluation of colorectal liver metastases

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Purpose: It is essential to figure out the exact extent of colorectal liver metastases (CRLM) for planning appropriate treatment strategy. Although gadoteric acid-enhanced MRI in combination of diffusion-

weighted MRI (Gd-EOB-MRI/DWI) has become popular to evaluate CRLM, it is still controversial whether it should be applied in every patient with CRLM. We aimed to assess whether Gd-EOB-MRI/DWI could detect CRLM more accurately than contrast-enhanced CT (CE-CT).

Methods: From 2008 to 2014, 250 patients underwent hepatectomy with diagnosis of CRLM. Among them, 205 patients underwent both CE-CT and Gd-EOB-MRI/DWI preoperatively. How many of the surgically resected lesions had been detected by the images were retrospectively reviewed. Detection rate by intraoperative ultrasonography (IOUS) was also evaluated.

Results: Of 442 lesions surgically resected, 419 resected lesions were pathologically confirmed as CRLM. The sensitivity of detecting CRLM was 77 % (323/419) in CE-CT and 93 % (388/419) in Gd-EOB-MRI/DWI ($p < 0.01$). The sensitivity to detect the CRLM of 1–5 mm, 6–10 mm and 11–15 mm in diameter by CE-CT was 9.6 % (5/52), 47 % (26/55) and 76 % (57/75), respectively, whereas that by Gd-EOB-MRI/DWI was 54 % (28/52), 91 % (50/55) and 99 % (74/75), respectively, and the difference was significant ($p < 0.01$ in all three groups). Sensitivity by Gd-EOB-MRI/DWI or IOUS was 95 % (396/419), which was higher than Gd-EOB-MRI/DWI only ($p = 0.01$). [Conclusion] Gd-EOB-MRI/DWI could detect CRLM of especially small lesions ≤ 15 mm with higher sensitivity than CE-CT, therefore, is an essential modality for preoperative evaluation.

O-187

Intraoperative ICG measurement during major hepatectomy for HCC. Hype or hope?

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Background: Preoperative ICG measurement has been the gold standard for preoperative evaluation for liver resection. 3–8 % patients still develop liver failure after hepatectomy despite satisfactory assessment.

Objective: A direct measurement of future liver remnant (FLR) by ICG elimination should provide better outcome prediction.

Patients and methods: Patients who is undergoing major liver resection for HCC from 2010 were included to this study. The ICG retention rate of the future liver remnant was measured by LiMON pulse densitometry during major hepatectomy. After laparotomy, the ipsilateral inflow of the liver with tumour was controlled by vascular clamp temporarily. After intravenous injection of 0.5 mg/kg of ICG to the patient, the ICGR15 was evaluated by a separated team of doctors. The operating surgeons were blinded from the measurements.

Result: 50 patients 42 male and 8 females were included. All 50 patients had major hepatectomy for HCC. The median operation time was 343 min. The median blood loss was 0.8 L. 3 patients developed liver failure after the operation. One patient end up in mortality and one patient received rescue liver transplant. All of them had intraoperative ICGR15 more than 50 %. The risk of hospital mortality and postoperative morbidity increased from 0 to 33.3 % ($P = 0.061$) and from 19.1 to 100 % ($P = 0.013$) respectively if the intraoperative ICGR15 remains as high as 50 %.

Conclusion: Intraoperative ICG study is a direct functional evaluation of the future liver remnant. In additional to preoperative evaluation, it should be used as an additional measure to prevent postoperative liver failure.

O-188

Simulation and navigation using a 3D printed liver model in laparoscopic liver surgery**Yasuji Seyama, Hiroyuki Kanomata, Hiroki Kudo, Nobutaka Umekita**

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Introduction: Three-dimensional (3D) analysis has enabled preoperative simulation and intraoperative navigation in liver surgery. However, 3D images were demonstrated only on the 2D monitor. Recently, we made 3D liver models with vascular structures and tumors by a 3D printer, and used in complicated liver resections. Herein, we present simulation and navigation using 3D printed liver models in laparoscopic liver surgery.

Methods: Preoperative CT images were reconstructed in a 3D configuration, including the liver parenchyma, portal vein, hepatic vein, and tumors. A liver model with internal structures was made by a 3D printer. 3D liver models were made by the parts to be resected and to be preserved separately.

Results: 3D printed liver models were made in three cases of laparoscopic liver resection, partial resection of S5, S7, and S8. Preoperatively, the surgeons simulate surgical procedures using the 3D model for a hand and acquire a real image inside the liver. Intraoperatively, liver resection is performed according to navigation using the 3D liver model in a sterilization bag. In all cases, planned liver resections were successfully carried out by the simulation and navigation using 3D printed model.

Conclusion: A 3D printed liver model is useful in laparoscopic liver surgery for pre-operative simulation and intra-operative navigation.

O-189

Our novel way to insert sheet type materials at laparoscopic hepatectomy: Corkscrew insertion method**Youichi Kawano¹, Nobuhiko Taniai², Satoshi Matsumoto¹, Masato Yoshioka², Nobuyuki Sakurazawa¹, Tetsuya Shimizu², Akihisa Matsuda¹, Hiroshi Yoshida², Masao Miyashita¹, Eiji Uchida²**¹Department of Surgery, Nippon Medical School Chiba Hokusou Hospital, Chiba, Japan; ²Nippon Medical School, Tokyo, Japan

Background: To conduct safer Lap-H procedure, hemostatic procedure for massive bleeding must be important but it is sometimes difficult during laparoscopic procedure. During the open hepatectomy, we can easily apply TachosilR which is sheet type material with good hemostatic ability, while it can be difficult during Lap-H. Then, we developed Corkscrew insertion method (CI) which is the way to insert the TachosilR via laparoscopic port. And it also can adapt to insert SeprafilmR to prevent post-operative adhesion. We will introduce them using video.

Method: For TachosilR, we gently compress the TachosilR in package. We cut it to one eighth with lidded paper of its package. We hold the corner of them by laparoscopic forceps, and insert it via 12 mm in diameter laparoscopic port with pronation movement like using a corkscrew. We can also adapt the method for SeprafilmR. We cut the SeprafilmR CS type to a quarter. After that we make moist to the SeprafilmR and put it on the cover sheet. And, we insert them as same way as above mentioned.

Result: We not only can insert the TachosilR and SeprafilmR via laparoscopic port but attach them to intended place very easy even in Lap-H. Because, when they enter to the abdominal cavity, they automatically spread themselves because of their lidded and cover paper. It make the procedures very easy and we could stop the massive bleeding from IVC.

Conclusion: Corkscrew insertion method might be useful to stop bleeding or prevent adhesion in any kind of laparoscopic surgeries.

O-190

Impact of pretransplant sarcopenia on survival after liver transplantation: A prospective study**Toshimi Kaido¹, Yumiko Tamai², Yuhei Hamaguchi¹, Shinya Okumura¹, Atsushi Kobayashi¹, Shintaro Yagi¹, Nobuya Inagaki^{2,3}, Shinji Uemoto¹**¹Department of Hepato-Biliary-Pancreatic and Transplant Surgery, Kyoto University, Kyoto, Japan; ²Department of Metabolism and Clinical Nutrition, Kyoto University Hospital, Kyoto, Japan; ³Department of Diabetes, Endocrinology and Nutrition, Graduate School of Medicine, Kyoto University, Kyoto, Japan

Aims: Skeletal muscle depletion and decrease in muscle strength, referred to as sarcopenia, predicts morbidity and mortality in patients undergoing digestive surgery. However, posttransplant changes in these sarcopenic parameters are unclear. The present study prospectively investigated the impact of pretransplant sarcopenia on survival and sequential changes in sarcopenic parameters after living donor liver transplantation (LDLT).

Methods: Fifty-five consecutive adult patients who underwent LDLT at Kyoto University Hospital between January 2013 and December 2014 were enrolled in this study. Median patient age was 56 (range 21–68), 34 (62 %) were male, median MELD score was 19 (range 6–41). Sarcopenia was assessed by the measurement of skeletal muscle mass (SMM) obtained by a multifrequency body composition analyzer and handgrip strength. We defined patients with sarcopenia as those with low SMM (<90 % of the standard SMM) and low handgrip strength (<26 kg for men, <18 kg for women). The impact of pretransplant sarcopenia on short-term survival and sequential changes in sarcopenic parameters including SMM and muscle strength were analyzed.

Results: The overall survival rate in patients with sarcopenia (n = 9) was significantly lower than in patients without sarcopenia (n = 46) (p = 0.002). SMM worsened after LDLT and did not recover to preoperative levels until 1 year after LDLT. In contrast, handgrip strength recovered to preoperative levels at 6 months after LDLT following sharp decrease at 1 month after LDLT.

Conclusions: Pretransplant sarcopenia was closely involved with short-term survival after LDLT and the recovery of handgrip strength preceded that of SMM.

O-191

Patterns of Disability on the Liver Transplant waitlist: from the FrAILT Study**Jennifer Lai, Mariya_L Samoylova, Marta Haftek, Jennifer Lai**

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Background: In patients with cirrhosis, disability of activities of daily living (ADLs) and instrumental ADLs (IADLs) is associated with increased hospitalizations, length of stay, and higher mortality.

Methods: Outpatients >18 years listed for LT with laboratory MELD ≥ 12 at a single, high-volume, LT center in the U.S. underwent assessment of disability using two validated scales during clinic visits: (1) Activities of Daily Living (ADL), which includes 6 basic activities necessary to function within one's home, and (2) Instrumental ADL (IADL), which includes activities necessary to function within society.

Results: Of 681 LT candidates: 35 % were women, 51 % had chronic HCV, median [interquartile range (IQR)] age was 60 (53–64) years and MELD was 15 (12–18). Median (IQR) follow up time was 7.2 months (2.6–15.5). At first assessment, 65.9 % of patients had no ADL disabilities; 55.6 % had no IADL disabilities. Patients <65 years were not less likely to be disabled than those >65. The most prevalent ADL disabilities were continence (22.1 %), dressing (12.1 %), and transferring (11.3 %); the most prevalent IADL disabilities were shopping (28.4 %), food preparation (23.2 %) and medicine management (21.5 %). Among patients without disability at first visit, the first ADLs to be lost were most likely to be transferring (25 %) or continence (24 %). The first IADLs to be lost were shopping (23 %) or preparing meals (17 %).

Conclusions: Deficits of both basic and instrumental ADLs are highly prevalent in cirrhotics listed for LT. Further work will evaluate the predictive utility of functional disability for poor outcomes on the liver transplant waiting list.

O-192

Living donor liver transplantation for HIV/HCV coinfected recipients with hemophilia

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Patients with hemophilia are frequently infected with both hepatitis C virus (HCV) and human immunodeficiency virus (HIV). With the advance of antiretroviral treatment, end-stage liver disease (ESLD) due to HCV has become a major cause of death, and liver transplantation plays an important therapeutic role. 8 consecutive living-donor liver transplantations (LDLTs) for HCV-related ESLD/HIV coinfection with hemophilia were evaluated retrospectively. Grafts were seven with a right liver graft and one with a left liver graft. The donors were female in six and male in two recipients, with a mean age of 51 (35–61) years. All patients underwent antiretroviral therapy (ART) treatment and had a controlled viral load before LDLT. While preemptive anti-HCV treatment was started in all cases at a mean of 34 (10–57) days postoperatively, SVR was achieved in three cases (38 %). Postoperative ART was successfully started in seven patients at a mean of 44 (6–71) days postoperatively, resulting in a controlled HIV viral load and well-maintained CD4 count, while one died before starting ART treatment. The cumulative 1-, 3-, and 5-year survival rates with a mean follow-up period of 7.5 (1–14) years were 75, 75, and 56 %, respectively, although the long-term outcome was diminished due to difficulties treating HCV and HIV coinfection, especially

in early cases when the current antiviral drugs were unavailable. In conclusion, LDLT with recent advances in antiviral therapy for both HCV and HIV is acceptable for HIV/HCV coinfected patients with hemophilia.

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Daclatasvir and Asunaprevir for recurrent hepatitis C following living donor liver transplantation

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The management of recurrent hepatitis C virus (HCV) infection following liver transplantation (LT) remains a challenge: however the recent development of direct acting antivirals has been promising. We prospectively investigated the efficacy and safety of the anti-HCV therapy with daclatasvir (DCV) and asunaprevir (ASV) in nine patients undergoing living donor liver transplantation (LDLT) with biopsy-proven recurrent hepatitis due to HCV genotype 1b (median age was 64 and 3 were female), including 2 on hemodialysis and 1 with HIV coinfection. Eight of them were non-responder to the PEGylated-interferon plus ribavirin, the other one was diagnosed as fibrosing cholestatic hepatitis immediately post-LDLT. The resistant associated substitutions at neither L31 nor Y93 in the NS5A region of the HCV genome were detected in the 9 patients prior to the anti-HCV therapy with DCV/ASV. As for the immunosuppressive regimen, four had been on cyclosporine prior to the initiation of DCV/ASV which was switched to tacrolimus, and the other five remained on tacrolimus; the dose was successfully modified during the treatment. Five patients had completed the intended 24-week treatment course and all of them achieved undetectable viral load at the latest follow up (SVR 4–24). The other four were on the treatment at the latest follow up with undetectable viral load. No significant adverse events including rejection were observed in 9 patients. In conclusion, anti-HCV therapy with DCV and ASV was safe and effective in LDLT recipients with recurrent hepatitis C (genotype 1b), even in those who were previously regarded to be difficult-to-treat.

O-194

Rabies Outbreak Involving Four Transplant Recipients in Kuwait and Saudi Arabia

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Background: In December 2014, 4 organ recipients died in 2 countries.

Methods: Chart review. Serum and CSF detection of rabies-specific antibodies. Detection of rabies virus antigen. Phylogenetic analysis compared virus from the recipients with variants circulating in Middle East and Asia.

Results: A 28 year old donor presented with chest infection, acute respiratory distress syndrome, cardiac arrest, and seizure. CT of the head was unremarkable. CSF culture was negative. A kidney recipient developed encephalitis within 2 months of transplantation and died. The second kidney recipient developed symptoms a week later and died. The family of the donor confirmed that he was bitten by a domestic dog in India 2 months prior to his death and did not receive prophylaxis; the dog died. The heart recipient had died after cardiac arrest with prodromal neuropsychiatric symptoms. The liver recipient developed drooling and hydrophobia, was treated with the Milwaukee protocol, and died after 34 days. Ante mortem brain biopsy showed Negri bodies. Rabies RNA was detected in brain and saliva. Rabies antibodies were detected in serum and CSF. Rabies was confirmed in the donor by RT-PCR of explanted cornea. The whole N gene sequences of rabies virus from recipient and donor were 99 % identical, belonging to the clade of rabies virus variants circulating in India. Deceased kidney recipients had no rabies antibodies. Corneas were explanted from 2 recipients who received prophylaxis; they remain well.

Conclusion: Avoidance of transmission of rare pathogens requires wide bandwidth diagnostics and rapid-cycle surveillance within transplantation networks.

O-195

Long-Term HBV Prophylaxis With Oral Anti-virals After HBIg Discontinuation

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Combination prophylaxis with hepatitis B immune globulin (HBIG) plus an oral anti-viral (OAV) is widely accepted regimen for the prevention of recurrent hepatitis B virus (HBV) infection after liver transplantation, although there is no consensus on dose and duration of HBIg. Because of the risk of persistency of HBV infection in allograft tissue, there is an ongoing debate about long-term prophylaxis.

Methods: One-hundred and fifty (116 male, 34 female) patients who had HBIg + Lam prophylaxis and who have no detectable serum virologic markers (HBsAg and/or HBV DNA) for recurrent disease during follow up of at least 3-years were included study. Histologic examination is also performed.

Results: Mean age was 47-years (18–64 years). Median duration of follow up was 5 (3–9) years after transplantation. Sixty-four patient had delta co-infection and 44 patients had HCC before transplantation. Of the patients, 108 (72 %) had their grafts from living donors. The liver graft showed no hepatitis on histologic examination. HBIg discontinued and patients continued to take an OAV (lamivudine or telbivudine, or entecavir, or tenofovir). After a median 25 months follow up no patients taking entecavir or tenofovir experienced HBV recurrence (reappearance of serum HBsAg); However %22 recurrence was observed in lamivudine and telbivudine group.

Conclusion: After combination prophylaxis with HBIg + OAV, discontinuation of HBIg and continuing OAV monoprophyllaxis is safe with entecavir and tenofovir. But there is significant recurrence risk in case of lamivudine monoprophyllaxis.

O-196

Transplantation of hepatocyte like cells from human mesenchymal stem cells into F344-scld gamma rat

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Background: Hepatocyte transplantation has been considered as an attractive method for lethal liver failure, which could be an alternative to liver transplantation. However, this therapy still cannot resolve some issues. Recently, it was reported that mesenchymal stem cells (MSCs) have a potency to differentiate into hepatocytes. MSCs are expected to be a potential source for cell therapy without the risk of immune rejection and the difficulty of culture. The aim of this study is to investigate the transplantation of hepatocyte like cells (HLCs) from human MSCs into F344-scld gamma (FSG) rats.

Methods: We investigated the engraftment of HLCs from human bone marrow MSCs after transplantation into the livers of FSG rats. Two-week-old FSG rats were given intraperitoneal injections of retrorsine. Three days after retrorsine treatment, the HLCs were transplanted into the rats via the portal vein. To investigate the paracrine effect of transplanted HLCs, we compared apoptosis, proliferation of native liver cells and survivorship between the HLCs group and the undifferentiated MSC transplantation (uMSC) group after receiving acute liver injury.

Results: The transplanted cells were successfully and widely engrafted into recipients' livers. And, transplanted HLCs expressed mature liver marker. At 10 days after transplantation, the survival rate of HLCs group significantly improved in comparison to that of uMSC group. Moreover, they decreased the apoptosis and promoted the proliferation of native liver cells in HLCs group.

Conclusion: This study demonstrates the engraftment of the transplanted HLCs from human MSCs. The paracrine effect of HLCs is more effective than that of uMSCs.

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Promotion of liver transplant tolerance by hepatic plasmacytoid dendritic cells

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Background: The liver is tolerogenic organ that is accepted without immunosuppressive therapy when transplanted across MHC barriers in mice. Although plasmacytoid dendritic cells (pDC) are a main source of interferon- α , liver pDC display tolerogenic properties and contribute to oral tolerance. Here we hypothesized that liver pDC might play an important role in immune regulation and the promotion of liver transplant tolerance.

Methods: Liver pDC were depleted from 10–12 week-old male C57BL/6 (B6; H2b) mice by intravenous injection of mPDCA (120G8) mAb. Normal or pDC-depleted B6 livers were transplanted orthotopically into normal 10–12 week-old male C3H (H2k) mice. Graft rejection was assessed by recipient survival and serum ALT levels and histology on day 4 after transplantation. We also assessed the impact of pDC on alloimmunity using a delayed-type hypersensitivity (DTH) model.

Results: Whereas mice transplanted with normal livers ($n = 6$) survived more than 100 days without any immunosuppression, 83 % ($n = 5/6$) mice that received pDC-depleted livers died within 55 days (MST = median survival time: normal: >100 days vs pDC-depleted 25 days; $p < 0.01$). Serum ALT at day 4 post LTx was significantly higher in mice given pDC-depleted livers than in those given normal livers (untreated: 115 ± 35 IU/l vs pDC-depleted 885 ± 21 IU/l; $p = 0.0014$). pDC-depleted liver allografts displayed more severe liver injury compared with normal grafts. pDC-depleted splenocytes induced enhanced DTH response compared with splenocytes without pDC depletion.

Conclusions: Donor pDC depletion induces stronger allogeneic T effector cell responses and breaks liver transplant tolerance. Donor liver pDC may play an important in tolerance induction following liver transplantation.

O-198

B cell and lineage negative cell chimerism induce bad outcome in post transplantation hepatitis C

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Background: The donor liver resident immune cells could be transferred to recipient. We analyzed perfusate cells, and detected them after post orthotopic transplantation in hepatitis C (OLT-CHC) patients.

Methods: Liver perfusates and peripheral blood were collected from 20 human donor liver grafts. Cells were stained to detect monocytes, B cell, CD4 + T cell, CD8 + T cell, NK cell, lineage negative cells, plasmacytoid dendritic cell (pDC), myeloid DC (mDC), regulatory T (Treg) and type 1 regulatory T (Tr1). To reveal the perfusate involvement in recipient, we studied peripheral blood donor cell chimerism in human leucocyte antigen (HLA) mismatched 18 patients [(post-transplantation hepatitis C recurrence (OLT-CHC) 8, HCV carrier with persistently normal ALT (OLT-PNALT) 3, and Control 7)].

Results: The perfusate and peripheral cell analysis revealed that the frequency of CD8 + T cells (P22.2 %, B16.5 %), monocytes (P0.932 %, B0.068 %), NK cells (P83.1 %, B64.1 %), pDC (P7.33 %, B1.94 %) and mDC (P6.35 %, B0.932 %) were increased and Treg (P3.27 %, B4.95 %) was decreased in perfusate compared with peripheral blood. The Tr1 cells were lower in the perfusate (P0.35 %, B1.52 %). The chimerism early after transplantation was

found in CD14 + cells (0.8 %), CD8 + T cells (1.7 %), CD4 + T cells (1.5 %), CD19 + B cells (0.95 %) and NK cell (4.5 %) as the perfusate dominant pattern likely. The chimerism of CD19 + B cells and CD56 negative cells, were significantly more in the OLT-CHC compared with in the OLT-PNALT.

Conclusion: The perfusate indicated the specific phenotype compared with peripheral blood. The B cells and lineage negative cells early chimerism after OLT correlated with hepatitis C recurrence.

O-199

Expression of activation-induced cytidine deaminase in hepatocytes under immune response against HBV

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Aims: Recently, a novel mechanism of anti-HBV immunity has taken the spotlight, the potent cellular defense achieved by APOBEC proteins. Activation-induced cytidine deaminase (AID) is one of the APOBEC family members and could suppress HBV replication in hepatocytes. The aim of the present study is to determine cytokines and intrahepatocytic mediators including AID that could contribute to intracellular anti-HBV immunity using a mouse model that recapitulates the immune response against HBV.

Methods: Wild-type Balb/c mice were administered plasmids encoding HBsAg with hydrodynamic tail vein injection. Some mice were immunized with recombinant HBsAg before hydrodynamic injection. Immune responses were evaluated serologically and histologically, and comprehensive expression analyses were performed using frozen liver specimens.

Results: Histologic examination revealed liver inflammation following the administration of HBsAg after preimmunization, but not after HBsAg administration without preimmunization. Findings from serum HBsAg and anti-HBs evaluation supported the association of hepatic inflammation with the HBsAg-specific immune response in HBsAg-administered mice after preimmunization. Expression analyses revealed the upregulation of several cytokines, including tumor necrosis factor- α in the liver of HBsAg-administered mice after preimmunization. AID is upregulated by tumor necrosis factor- α stimulation via NF- κ B activation in hepatocytes. Furthermore, the reporter mice model clearly revealed that AID expression was induced in hepatocytes under the immune response against HBV.

Conclusion: These results suggest that AID expression is induced in hepatocytes in response to an acquired immune response against HBV, and support the putative role of AID in intracellular anti-HBV immunity.

O-200

Fibronectin restricts TGF-beta bioavailability and matrix remodeling in chronic liver fibrogenesis

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Fibrosis is characterized by extracellular matrix (ECM) remodeling and stiffening. However, functional contribution of tissue stiffening to non-cancer pathogenesis remains largely unknown. Fibronectin (Fn)

is an ECM glycoprotein substantially expressed during tissue repair. We have previously demonstrated using a mouse model lacking Fn that Fn-null livers do not interfere with reconstruction and resolution of collagen organization in initial stages of liver damage (*Gastroenterology*, 2011). Here we show in advanced chronic liver fibrogenesis that, unexpectedly, Fn-null livers lead to more extensive liver cirrhosis, which is accompanied by increased liver tissue stiffness and deteriorated hepatic functions. Furthermore, Fn-null livers exhibit more myofibroblast phenotypes, and accumulate highly disorganized/diffuse collagenous ECM composed of thinner and significantly increased number of collagen fibrils during advanced chronic liver damage. Mechanistically, mutant livers show elevated local TGF-beta activity and lysyl oxidase expressions. A significant amount of active lysyl oxidase is released in Fn-null hepatic stellate cells in response to TGF-beta1 through canonical and non-canonical Smad such as PI3 kinase-mediated pathways. TGF-beta1-induced collagen fibril stiffness in Fn-null hepatic stellate cells is significantly higher compared to wild-type cells. Inhibition of lysyl oxidase significantly reduces collagen fibril stiffness, and treatment of Fn recovers collagen fibril stiffness to wild-type levels. Thus, our findings indicate an indispensable role for Fn in chronic liver fibrosis/cirrhosis in negatively regulating TGF-beta bioavailability, which in turn modulates ECM remodeling and stiffening, and consequently preserves adult organ functions. This regulatory mechanism by Fn could be translated for a potential therapeutic target in broader variety of chronic fibrotic diseases.

O-201

WFA(+)-M2BP interacts with Kupffer Cells which activates Hepatic stellate cells

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Background: Non-invasive evaluations of liver fibrosis have been a challenge for a long time, and recently, a serum marker, Wisteria floribunda agglutinin-positive Mac-2 binding protein (WFA(+)-M2BP) reflecting liver fibrosis was identified. The unique characteristics attracts attentions such as the facts that serum WFA(+)-M2BP rapidly decreases after SVR of HCV infection and serum WFA(+)-M2BP predicts carcinogenesis of HCCs. However the mechanism and function of increased serum levels of WFA(+)-M2BP in the patients with liver fibrosis remained to be elucidated.

Materials and Methods: We used liver tissues and isolated cell subpopulations of liver samples taken from 10 patients underwent liver resections (5 for HCCs and other 5 for metastatic liver tumors) and 5 patients underwent liver transplantation.

Results: 1. Supernatant of cultured cell subpopulations showed the source of WFA(+)-M2BP was Hepatic stellate cells (HSCs) using sandwich ELISA. The levels of secreted WFA(+)-M2BP increased after in vitro activation of HSCs. 2. Tissue section staining showed WFA(+)-M2BP positive cells merged with CD 68 [Kupfer cells (KCs)], and positive cells increased depends on the severity of liver fibrosis. 3. Co-culturing HSCs and KCs dramatically increased α -SMA expression evaluated by western blotting, which indicates pro-fibrotic properties of KCs. Interestingly, the increased production of α -SMA by HSCs was dramatically inhibited when lactose, M2BP inhibitor, was applied during co-culture medium. In conclusion, our data reveal that WFA(+)-M2BP secreted from HSCs are key factor to mediate

KCs pro-fibrotic properties which activates HSCs. The spiral between HSCs and KCs may leads to liver fibrosis via WFA(+)-M2BP.

O-202

Development of Effective Gene Therapy for Liver Fibrosis

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Background & Aims: While diagnostic strategies for liver fibrosis have been established, no standard therapy for liver fibrosis has been developed. The objective of this study was to assess the approach of matrix metalloproteinase gene delivery to prevent liver fibrosis progression.

Methods: The rat liver fibrosis model was developed by the bile duct ligation procedure, followed by liver-targeted hydrodynamic gene delivery of a matrix metalloproteinase-13 gene (MMP13) expression vector. The serum MMP13 concentration was examined to determine the efficiency of gene delivery. Hyaluronic acid was used as a serum marker to determine the therapeutic effect of MMP13 gene therapy on liver fibrosis, and histological analyses including sirius red staining were performed to assess the degree of liver fibrosis after the gene therapy.

Results: The serum level of MMP13 peaked at 71.7 pg/ml in the MMP13-treated group 14 days after the gene therapy, whereas the non-treated group only showed a level of approximately 5 pg/ml ($p < 0.001$). These levels were sustained for the next 70 days. The statistically lower level of the hyaluronic acids in the treated group versus the non-treated group ($p < 0.05$), reveals the therapeutic effect of MMP13 gene delivery on liver fibrosis. Quantitative analysis of fibrotic tissue stained with sirius red also showed a statistically larger volume of fibrotic tissue in the non-treated group compared to that of MMP13 treated rats ($p < 0.05$).

Conclusion: These results suggest that the liver-targeted hydrodynamic delivery of the MMP13 gene may be effective in the prevention of liver fibrosis.

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Augmentation of hepatic progenitor cells-mediated liver regeneration by antifibrotic therapy

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Hepatic progenitor cells (HPC) rescues liver regeneration on diseased liver. It is commonly known that hepatic stellate cells (HSC) play important roles as niche component cells of HPC, and that HPC expands along with HSC activation and liver fibrosis development. However, less is known about whether liver fibrosis development affects HPC-mediated regeneration. We have reported anti-fibrotic

effect of angiotensin 2 receptor blocker 1 (ARB) on diseased liver by inhibition of HSC activation. Our current study was aimed to elucidate interactions between HSC and HPC-mediated liver regeneration using ARB on a DDC-induced mouse liver model. DDC-treatment augmented liver injury with fibrosis development and HPC expansion. ARB-treatment attenuated α -SMA-positive activated HSC with increased hepatocyte (HC) differentiation of HPC, which may result in gain of liver/body weight ratio. Our in vitro experiments showed that ARB treatment did not alter capacity for HPC differentiation. In contrast, co-culture of HPC and human HSC line (LX-2) augmented biliary epithelial cell (BEC) differentiation of HPC but attenuated efficiency for hepatocyte differentiation. Treatment with angiotensin 2 enhanced this polarity of HPC differentiation towards BEC, which was blocked by ARB. These results indicated that HSC augments HPC differentiation fates towards BEC. We also confirmed that both LX-2 and primary activated HSC exclusively express Jagged1, a ligand for NOTCH, which plays central role in HPC differentiation fate towards BEC. Anti-fibrosis treatment may also be beneficial on efficient HPC-mediated liver regeneration via preferential differentiation of HPC towards HC by blocking NOTCH pathway in HPC niche.

O-204

Ex vivo expanded circulating CD34⁺ cells exhibit potent therapeutic effect on liver cirrhosis

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Ex vivo expansion of autologous cells is indispensable for cell transplantation therapy of patients with chronic disease including liver cirrhosis. The aim of this study was to investigate the efficacy of human ex vivo expanded granulocyte-colony stimulating factor (G-CSF)-mobilized peripheral blood CD34⁺ cells for treatment of cirrhotic rat liver. Recipient rats were intraperitoneally injected with CCl₄ twice weekly for 3 weeks before administration of CD34⁺ cells. CCl₄ was then re-administered twice weekly for 3 more weeks, and the rats were sacrificed. Saline, non-expanded or expanded CD34⁺ cells were injected via the spleen. After seven days in culture, G-CSF-mobilized CD34⁺ cells were effectively expanded in a serum-free culture medium. Expanded CD34⁺ cells were also increasingly positive for cell surface markers of VE-cadherin, KDR and Tie-2, whereas they were down-regulated for CD34, CD133 and CD117 as determined by flow cytometric analysis. The expression of pro-angiogenic growth factors and adhesion molecules in expanded CD34⁺ cells increased compared with non-expanded CD34⁺ cells. Expanded CD34⁺ cell transplantation reduced liver fibrosis, with a decrease of type-I collagen and α SMA⁺ cells after 6 weeks of CCl₄ treatment. Assessments of hepatocyte and sinusoidal endothelial cell proliferative activity indicated the superior potency of expanded CD34⁺ cells over non-expanded CD34⁺ cells. Integrin β 3 was the most up-regulated gene in the culture. The inhibition of integrin α v β 3 and α v β 5 disturbed the engraftment of transplanted CD34⁺ cells and aggravated liver fibrosis. These findings strongly suggest that expanded CD34⁺ cells enhanced the therapeutic efficacy of cell transplantation in a rat cirrhotic model.

O-205

A new strategy for liver tissue fabrication and transplantation.

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Although previous demonstrations of whether engineered liver tissue may engraft and function in vivo was performed in variety of ectopic sites, considering the hepatic nature of growing with portal blood and excretion of bile acid, transplantation should be performed to liver itself. Here we show a new strategy for liver tissue fabrication and transplantation. We first established the reproducible formation of multicellular spheroids containing human hepatocytes, human umbilical vein endothelial cells and human mesenchymal stem cells. We fabricated liver tissue fusing the multicellular spheroids using “Bio-3D printer”. This system was assembling spheroids into a three-dimensional shape on needles. After the spheroids were fused with each other, the needles were removed and a scaffold-free 3D tissue was obtained. Histological examination of the biofabricated liver tissue showed reticular endothelial network with production of extracellular matrix and cell migration in vitro. Next we developed a new transplantation method for liver tissue, implantation onto the bare parenchyma at the transection plane of liver. Transplantation of fetal rat liver onto the transection plane showed growth of the graft and expressed longer function rather than that into mesenterium, on naïve liver surface and into liver parenchyma. This result indicated the sufficient portal supply from transection plane to the graft. Finally the biofabricated liver tissues were transplanted onto the transection plane of nude rats. The result showed engraftment and functioning of the transplanted liver tissues. As a conclusion, this strategy may provide a novel liver tissue fabrication technologies for liver regeneration field.

O-206

The new drug, Sodium-glucose cotransporter-2(SGLT2) inhibitor prevents liver fibrosis and cancer

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Background: Liver fibrosis caused by Hepatitis B, C and NASH and can progress to refractory state and to HCC. Sodium-Glucose cotransporter-2 <SGLT2> inhibitor is a new oral drug which developed for the treatment of type 2 diabetic patients by rational drug design. The aim of this study is to investigate whether SGLT-2

inhibitor has any effects on the development of liver fibrosis as well as preneoplastic lesions.

Methods: The effects of the SGLT-2 inhibitors were examined using the choline-deficient L-amino acid-defined <CDAA> diet-induced liver fibrosis model. Diethylnitrosamine <DEN> 10 mg/kg was injected intraperitoneally once a week. One group received CDAA diet containing SGLT-2 inhibitor <10 mg/kg/day > and DEN injection, the other group received CDAA diet and DEN injection without SGLT-2 inhibitor. Liver fibrosis was analyzed by Azan, Sirius-red, α SMA, ED-1 expression. Development of preneoplastic lesions was assessed by glutathione S-transferase placental form <GST-P> expression. Type I procollagen, TIMP1, TIMP2, TGF β R1, MMP2, 9, 13, AFP, TNF α , MCP1, EpCAM mRNA expression were analyzed using RT-PCR and DNA array.

Results: After 16 weeks, SGLT-2 inhibitor prevented liver fibrosis by Azan, Sirius-red expression <p <0.05>. ED1 expression was decreased <p <0.05>. SGLT-2 inhibitor reduced the area of GST-P positive lesions as preneoplastic lesions <p <0.05>. Administration of SGLT-2 inhibitor significantly reduced levels of serum AST <mean value: SGLT-2 inhibitors 309.6 vs Control 391.3 IU/l, p <0.05>, serum albumin <mean value: SGLT-2 inhibitors 4.6 vs Control 3.9 g/dl, p <0.05>. SGLT-2 inhibitor significantly inhibited Type 1 procollagen, TGF β , TIMP2, MCP1, AFP mRNA expression <all of p <0.05>.

Conclusion: SGLT-2 inhibitor prevented liver fibrosis and preneoplastic lesions. We suggested that SGLT-2 inhibitor will be the new drug of inhibiting liver fibrosis with impaired glucose tolerance as pre-emptive medical care.

O-207

Identification of mechanism regulating characters of stem/progenitor cells during liver development.

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Background: Fetal and adult liver progenitor cells (LPCs) have high proliferative ability and can differentiate into both hepatocytic and cholangiocytic cells. Adult LPCs but not fetal LPCs could form cysts with cholangiocytic characters in 3 dimensional matrices gel culture. Thus, some important changes that induce fetal LPCs into adult LPC-like progenitor cells might occur during liver development. In this study, we aimed to identify the molecular mechanism regulating these changes of murine LPCs.

Method: Dlk⁺ murine fetal LPCs were purified by magnet beads (primary cells). Primary cells were seeded; directly into 3D matrices gel, to 2D plate culture for 7 days and to 2D plate culture with cytokines such as Oncostatin M (OSM) and hepatocyte growth factor (HGF) for 7 days. Cells, which were pre-cultured on 2D gelatin-coated plates (Cultured cells), were seeded into 3D matrices gels.

Results: After 2D pre-culture of fetal LPCs, these cells could form Albumin⁺Cytokeratin19⁺ cholangiocytic cysts in 3D matrices gels. Formed cysts included Ki-67⁺ cells and could expand for more than 6 months in vitro. In contrast, adding either OSM or HGF during 2D pre-culture led to decrease of cyst formation and down regulation of CD133⁺ cell fraction. Flow cytometric analyses revealed that CD133⁺CD326⁺ cells effectively formed cholangiocytic cysts. These results suggest that the 2D pre-culture on gelatin coated-plate change

characteristics of fetal LPCs into adult LPC-like progenitor cells and these changes were inhibited by the stimulation of OSM and HGF.

O-208

Effect of IFN on TLR-Associated Gene Expression in Hepatoma Cells with Integrated HBV DNA

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Background and Aim: Eliminating serum HBV surface antigen (HBsAg) is short term goal in clinical management of HBV. The effects of HBV treatment on toll-like receptor (TLR)-associated gene expression remain unclear. We examined the effects of interferon (IFN) and nucleic acid analogues (NAs) on the production of HBsAg in human hepatoma cells. We also investigated the effects of IFN on TLR-associated gene expression to develop new therapeutic targets. **Methods:** HepG2.2.15, Huh1 and PLC/PRF/5 were treated with IFN and NAs such as Lamivudine, Adefovir, Entecavir and Tenofovir. HBsAg levels in the cell culture supernatants were determined by CLEIA. Then cellular RNA was extracted, cDNA was synthesized, and TLR-associated genes were measured by real-time RT-PCR. Methylation status of HBsAg-promoter regions were analyzed by bisulfite sequencing.

Results: HBsAg levels in the cell culture supernatants were decreased in Huh1 and PLC/PRF/5 by only IFN treatment. However, HBsAg levels were decreased by all NAs but not IFN in HepG2.2.15, in which HBV DNA replicates. We observed the upregulation of IL8, TLR2 and IP10 mRNA in both Huh1 and PLC/PRF/5. We did not observe the differences of methylation status of HBsAg-promoter regions among these cell lines.

Conclusion: IL8, TLR2 and IP10 play a role in the HBsAg disappearance and may be candidates in the treatment in HBV.

O-209

Integrative analyses of multi-omics data of Histone demethylase LSD1/2 in liver cancer cells

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Background and Aim: Most common liver diseases and liver cancers involve metabolic dysfunctions induced by acquired factors such as accumulation of metabolic stress, which is suggesting underlying epigenetic alterations of gene expression in response to cellular metabolic state. However, epigenetic mechanisms in their pathogenesis are unknown. Here, we focus on histone demethylase LSD1 and its family enzyme LSD2, both are related to carcinogenesis and metabolic regulation. To investigate how LSD1/2 regulate gene expression and cellular metabolism in the liver, we performed multi-

omics analyses using transcriptome and metabolome in HepG2 cell, a hepatocellular carcinoma (HCC) cell line.

Result and Discussion: An integrated transcriptome and metabolome analysis in LSD1-knockdown HepG2 cells using specific siRNA demonstrates that LSD1 activates glycolytic metabolism with a concurrent inactivation of mitochondrial respiration, thereby causes the metabolic state switch known as the Warburg effect, a typical metabolism of glycolysis independent on oxygen state in cancer cells. In addition, a similar analysis in LSD2-knockdown HepG2 cells indicates that LSD2 might be required for the repression of lipid metabolism genes, and for the proper control of intracellular lipid level. Moreover, we found both LSD1/2 are overexpressed in human HCC tissues by immuno-histochemical studies. Thus, these results suggest that, in HCC cells, LSD1 might mediate energy production through activating glycolysis and repressing mitochondrial respiration, and LSD2 could reduce the dependence on mitochondrial respiration through suppressing fatty acid uptake. We propose an integrative hepatic cancer metabolism map, in which LSD1/2 epigenetically coordinate expression of energy metabolism genes in HCC cells.

O-210

Inhibition of CBP/ β -catenin reduces liver fibrosis in mice

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Wnt/ β -catenin is implicated in organ fibrosis. However, the role of β -catenin-mediated signaling on liver fibrosis remains unclear. To explore this issue, the effects of PRI-724, a selective inhibitor of the cAMP-response element-binding protein-binding protein (CBP)/ β -catenin interaction, on liver fibrosis were examined using carbon tetrachloride (CCl₄)- or bile duct ligation (BDL)-induced mouse liver fibrosis models. Following repetitive CCl₄ administrations, the nuclear translocation of β -catenin was observed only in the non-parenchymal cells in the liver. PRI-724 treatment reduced the fibrosis induced by CCl₄ or BDL, accompanied by the suppression of S100A4 expression, a CBP/ β -catenin transcript. C-82, an active form of PRI-724, inhibited the activation of isolated primary mouse quiescent hepatic stellate cells (HSCs). These results suggest that the inhibition of CBP/ β -catenin suppresses liver fibrosis through the inhibition of HSCs activation. Thus, targeting the CBP/ β -catenin interaction may become a new therapeutic strategy in treating liver fibrosis.

O-211

Elastin derived peptide facilitates liver fibrosis regression via promoting aHSCs reversion

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Background: Elastin has been linked to maturity of liver fibrosis; however, its degradation in liver fibrosis regression has not been characterized. We investigated here the contribution of elastin-derived peptides (ED). degradation products of elastin, the main component of elastic fibers in activated stellate cell reversion.

Methods: We examined the expression levels of EDP in regression liver tissues by specific ELISA. We used 500 μ g/ml EDP to stimulate HSCs directly in serum-free medium for 24 h, and observed the influence of EDP on fibrogenesis.

Results: First, in response to reversion of liver fibrosis, levels of EDP gradually increased. Secondly, EDP directly decreased production of elastin, TIMP-1, collagen type I in hepatic stellate cells by deactivated the hepatic stellate cell. Further, mechanistically, deletion of receptor of elastin derived peptide reversed the deactivation response, resulting in increased collagen production and a-smooth muscle actin expression in the activated HSCs.

Conclusion: Elastin derived peptide attenuates deactivation of hepatic stellate cell and the reversion of liver fibrosis. Thus, elastin derived peptide can be regarded as a potential therapeutic for liver fibrosis.

O-212

Macrophage β -catenin is critical to regulate innate immunity in liver inflammatory injury

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It has been shown that β -catenin is an important regulator of cell development and regeneration. Our previous studies demonstrated the key role of β -catenin in the regulation of immune response at the innate-adaptive interface by controlling of dendritic cell (DC) programs in hepatic ischemia and reperfusion injury (IRI). However, it is unknown how β -catenin may affect macrophage-mediated innate immune response during the course of hepatic IRI. This study was designed to dissect the molecular mechanisms of β -catenin in regulating innate immunity in macrophages.

Methods: The macrophage specific ablation of β -catenin (β -catenin^{M-KO}) was generated by crossing β -catenin transgenic (β -catenin^{flox/flox}) mice with LysM-Cre transgenic mice. Bone marrow-derived macrophages (BMMs) from β -catenin^{M-KO} and β -catenin^{flox/flox} mice were transfected with β -catenin-expressing vector (p β -catenin) or PPAR- γ siRNA/ROCK1 siRNA (100 nM), and then cultured for 24 h followed by additional 6 h of LPS (100 ng/ml) stimulation.

Results: Myeloid-specific β -catenin deletion (β -catenin^{M-KO}) increased the expression of ROCK1, PTEN, and TLR4. However, transfection of p β -catenin in LPS-stimulated BMMs decreased ROCK1 expression yet promoted PPAR- γ activation, increased Stat6 phosphorylation and PGC-1 β expression. Disruption of PPAR- γ with siRNA treatment in p β -catenin-transfected cells increased ROCK and PTEN activity. Furthermore, ROCK1 siRNA knockdown enhanced PTEN function leading to triggering TLR4/NF- κ B activation, which accompanied by increased expression of proinflammatory mediators.

Conclusion: This study demonstrates that β -catenin regulates macrophage-mediated innate inflammatory responses by promoting PPAR- γ and depressing ROCK/PTEN activity, leading to inhibiting TLR4/NF- κ B activation. Our novel findings imply a novel therapeutic potential for the management of liver IRI in transplant recipients.

O-213

Evidences of antifibrotic effect of the growth hormone releasing peptide 6**Gerardo E. Guillen, Jorge Berlanga, Ariana Garcia, Yssel Mendoza**

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Tissue fibrosis is a leading cause of morbidity and mortality. Current treatments for conditions such as hepatic fibrosis have been unsuccessful. The growth hormone releasing peptide 6 (GHRP6) is endowed with cardioprotective actions but its antifibrotic effect had not been anticipated. We examined the GHRP6 ability to prevent and revert liver cirrhosis after induction in Wistar rats by a subcutaneous administration of CCl₄. GHRP6 effects were examined after concomitant and delayed administration to toxic respectively. The percentages of hepatic fat, fibrosis, nodularity and septae thickness were histologically and morphometrically determined. Ascitis and portal dilation were judged by ultrasound and serum biochemical profile and oxidative stress parameters determined. Mechanistic involvement of selective gene/proteins was assessed by RT-PCR and immunohistochemistry. Microarrays showed gene expression profiles of GHRP6-treated liver samples on CapitalBio Rat Genome Oligo Array. GHRP6 concomitant intervention prevented in more than 85 % parenchymal fibrotic induration and therapeutic administration for only 15 days allowed for 37 % fibrotic clearance with more than 30 % reduction of septae thickness. The 60 days GHRP6 administration scheme produced a 75 % reduction of the fibrotic area with more than 60 % reduction of nodularity. GHRP6 reduced oxidative damage enhancing the activity of antioxidant enzymes. Vimentin and alpha smooth muscle actin immunodetection profile indicated GHRP6 reduced the number of activated stellate cells. GHRP6 administration reduced fibrogenic factors as TGF Beta and CTGF on Kupffer cells. These evidences suggest GHRP6 may control the liver fibroplastic response.

O-214

Cytoglobin deficiency enhances liver injury and fibrogenesis during cholestasis in mice**Tuong Thi Van Thuy¹, Le Thi Thanh Thuy¹, Katsutoshi Yoshizato², Norifumi Kawada¹**¹Department of Hepatology, Graduate School of Medicine, Osaka City University, Osaka, Japan; ²Academic Advisor Office, PhoenixBio Co. Ltd., Hiroshima, Japan

Aims: In this study we investigated the role of Cygb in the development of cholestasis-induced hepatic injury using Cygb knockout (KO) mice.

Methods: Cholestasis was induced in KO male mice and wild-type (WT) controls by bile duct ligation (BDL) with 4–8 mice for each group. Mice were killed at 24, 48, 72 h after BDL in a short-term study and 1, 2, 3 weeks in a long-term study.

Results: KO mice in the short-term study showed severe biliary infraction with increased level of ALT (695.8 ± 276.7 IU/L vs. 367.6 ± 259.2 IU/L in WT mice, $p < 0.05$), bilirubin (460.6 ± 113.5 mmol/L vs. 133.7 ± 49.6 mmol/L, $p < 0.001$) and bile acid (1150.4 ± 493.1 IU/L vs. 541.2 ± 199.6 IU/L, $p < 0.05$). Inflammatory reactions were increased in KO mice as evidenced by the increase of infiltrated inflammatory cells: neutrophils (29.3 ± 11.7 vs. 0.1 ± 0.1 cells/field in WT, $p < 0.001$); macrophages (34.8 ± 2.1 vs. 18.5 ± 1.9 cells/field, $p < 0.001$), and increased mRNA level of chemokines: *Cxcl2* 4.2-fold; *Cxcl5* 7.2-fold,

$p < 0.05$). Imbalance of oxidative stress and antioxidant was demonstrated by up-regulated pro-oxidant genes (*HO-1* 2.0-fold, $p < 0.001$) and lipid peroxidase (*MDA* 4.5-fold, $p < 0.0001$), while down-regulated antioxidant genes (*Catalase-1* 0.5-fold; *glutathione peroxidase* 4 0.5-fold, $p < 0.05$), in KO mice compared with WT counterparts. In the long-term study protein and m-RNA levels of fibrosis markers significantly increased in KO mice: collagen1 α -1 3.8-fold; α -SMA 4.0-fold, $p < 0.05$.

Conclusion: Cygb deficiency enhances liver injury and inflammation from early time points after BDL, and then later fibrogenesis.

O-215

Periostin promotes liver fibrosis by activating hepatic stellate cell via interacting integrins**Akiko Sugiyama¹, Keishi Kanno², Nobusuke Kishikawa², Norihisa Nishimichi³, Yasuyuki Yakosaki³, Susumu Tazuma^{1,2}**¹Department of General Internal Medicine, Graduate School of Medical Science, Hiroshima University, Hiroshima, Japan; ²General Internal Medicine, Hiroshima University Hospital, Hiroshima, Japan; ³Cell-Matrix Frontier Laboratory, Biomedical Research Unit, Hiroshima University, Hiroshima, Japan

Background & Aim: Periostin (PN), a matricellular protein, is required for tissue fibrosis and remodeling in heart, lung and skin. However, the participation of PN in liver fibrosis has not been fully elucidated. Our previous *in vitro* study has demonstrated significant role of PN in the activation of hepatic stellate cells (HSCs). This study investigates the effect of PN deletion on experimentally induced liver fibrosis *in vivo*. Furthermore, the influence of PN on HSCs behavior was investigated focusing on the interaction with integrins, known receptors for PN.

Methods: PN deficient (PN^{-/-}) and wild type (WT) mice were subjected to hepatotoxic (carbon tetrachloride; CCl₄, thioacetamide; TAA) or cholestatic (3.5-diethoxycarbonyl-1.4-dihydrocollidine; DDC) liver injury to compare the development of liver fibrosis of different etiology. To investigate the role of PN-integrin interaction in HSC function, we utilized LX2, a HSC cell line, in which the function of integrins was inhibited by neutralizing antibodies or gene silencing by siRNA.

Results: PN^{-/-} mice in three different liver fibrosis models developed negligible fibrotic lesions in contrast to extensive collagen deposition in WT mice. Consistently, the expression of fibrotic markers was reduced in PN^{-/-} mice. LX2 in the presence of neutralizing antibodies against integrin α v β 3 and α v β 5 resulted in significant decrease in fibrotic markers. In accordance, knock-down of integrin α v in LX2 on PN-coated plates dramatically inhibited cell attachment and reduced fibrotic markers at mRNA and protein levels.

Conclusion: These findings indicate that PN contributes to the development of liver fibrosis by enhancing HSCs activation and collagen production.

O-216

CYP2E1 inhibitor DDC upregulates MMP-13 expression *in vivo* through modulating miR-222**Tianhui Liu, Ping Wang, Min Cong, Lin Liu, Hufeng Xu, Jidong Jia, Hong You**

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Background & aims: We previously demonstrated that CYP2E1 inhibitor diethylthiocarbamate (DDC) significantly upregulated collagenase (MMP-1) expression in hepatic stellate cells (LX-2) *in vitro*. However, the mechanism was not elucidated. In this study, we investigated the regulation of MMP-13 by DDC *in vivo* and its possible molecular mechanisms.

Methods: C57BL/6 mice were treated with MCD diet (MCD group) or in combination with DDC (DDC group) by gastric perfusion (30 mg/kg, twice a week) for eight weeks. Liver samples were fixed, paraffin embedded, and sectioned. H&E and Masson staining were performed. Total protein, mRNA and miRNA were extracted from the liver tissue. The expression of miR-222 and MMP-13 were detected by realtime PCR.

Results: Compared with the control group (n = 8), serious hepatic steatosis, inflammation and mild fibrosis in the liver were seen by feeding MCD diet (n = 8) for 8 weeks. The expression of MMP-13 in the liver were higher in DDC treated group (14.9 ± 2.4 , n = 8) than MCD group (5.6 ± 3.1). Compared with the control group (1.2 ± 0.3), the expression of miR-222 in the liver significantly upregulated in MCD group (62.4 ± 12.3), while decreased sharply in DDC group (2.4 ± 1.3). In addition, the level of MMP-13 is negatively related to the miR-222 level.

Conclusions: DDC significantly downregulates the expression of miR-222, while upregulates the expression of MMP-13 in the liver of NASH model mice. The data indicates that miR-222 might be involved in the regulation of MMP-13 by DDC.

O-217

Exosomal microRNA panels as biomarkers predicting for progress of HBV-induced liver cirrhosis

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Aim: The objective of our study is to establish serum exosomal microRNA (miR) signatures to predict progression and reversion of hepatitis B (HBV)-induced cirrhosis undergoing anti-viral therapy.

Methods: Twelve HBV patients with compensated cirrhosis were selected based on their cirrhosis regression (decreased Fibroscan values of >5 kPa) or progression (increased or stable Fibroscan values) after 6 months of Entecavir treatment, respectively. Twelve matched healthy blood donors were used as a control group. Small RNA was isolated from purified exosomes and samples in each group were pooled and analyzed using a Human miRNome PCR array. Circulating exosomal miRNAs were isolated from 8 other compensated cirrhosis HBV patients to validate the change of selected exosomal miRNAs by qRT-PCR.

Results: Compared with control group, 59 out of 1066 miRNAs were up- or down-regulated at least 15-fold in regressed cirrhosis patients, with 46 out of 59 miRNAs showing correction (to more healthy) after therapy. MiR-582,-22,-7a,-889 and -3200 were the top 5 miRNAs which were significantly up-regulated before therapy and reversed almost to the normal level after therapy in regressed cirrhosis HBV patients. In progressed cirrhosis HBV patients, expression of miR-148b,-133,-4254,-3074 and -4288 were extremely decreased, and these miRNAs were less corrected as compared to the regressed patients. qRT-PCR analysis of exosomal miRNAs from 8 cirrhosis HBV patients showed that miR-93,-320d,-3176,-4272 and -4302 were significantly decreased in progressed patients before therapy.

Conclusions: Exosomal miRNAs signatures could be diagnostic or predictive for the HBV patients' clinical course of fibrosis regression or progression after anti-viral treatment.

O-218

Effects of the Mesenchymal Stem Cells on Common Bile Duct Ligated Rats

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Liver's regenerative capacity is interrupted with hepatic fibrosis. Cellular therapy is a promising approach that may preclude the need for OLT. Among them, mesenchymal stem cells (MSCs) have potential to Trans-differentiate into hepatic cells.

Aim: To show the effects of bone marrow MSCs transferred via the tail vein to the rats having hepatic fibrosis produced by common bile duct ligation (CBDL) model.

Method: Rats were divided into three groups; 1- CBDL rats that were given MSCs (CBDL + MSC), 2- CBDL rats given phosphate-buffered saline (PBS) (CBDL + PBS), 3- Healthy rats that were sham operated and given MSCs (Healthy + MSC). We analyzed the *in vivo* functional effects by glucose production, albumin secretion and serum ALT levels. MSCs were labeled with GFP to check the localization of stem cells and to get idea for the regenerative capacity in the injured liver. Splenocytes were isolated from spleen and cultured with Anti-CD3 and Anti CD28. Immunologic parameters were analysed with flowcytometry.

Results: Histologically, liver fibrosis developed in CBDL rats while the healthy rats group did not show any alteration in liver architecture. MSCs significantly suppressed the rats splenocyte proliferation more in CBDL + MSC compared with CBDL + PBS group. NK cells in peripheral blood significantly increased more in CBDL + MSC compared with CBDL + PBS. Peripheral CD4 + CD25 + ratio increased in CBDL + PBS compared with CBDL + MSC. MSCs significantly suppressed the TNF-alpha and IL-1 beta proinflammatory levels in CBDL + MSC compared with CBDL + PBS.

Discussion: Our findings suggest bone marrow derived MSC injection treatment may appear promising in liver injury and future clinical therapies are warranted.

O-219

Lower class 1 ADH and ALDH2 expression predict poorer outcome in male patients with liver cancer

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Background: The liver is the major organ for metabolizing ethanol mainly by class 1 ADH (ADH1) and ALDH2, which were found to be associated with several human malignancies.

Methods: Quantitative real-time PCR was performed to detect ADH1 and ALDH2 expression levels in tumor and adjacent tumor tissue specimens of 85 primary liver cancer (PLC) patients. Kaplan–Meier survival analysis was used to examine the influence of ADH1 and ALDH2 expressions on patients' survival. Additionally, by using chi-square test for $R \times C$ contingency table, the correlations between gene expressions and clinical data were tested.

Results: The levels of ADH1 and ALDH2 were lower in the tumor tissues compared to the adjacent non-tumor tissues ($p < 0.001$). The expression of ADH1 was positive correlated with ALDH2. Lower ADH1A, ALDH2 expressions either in tumor or non-tumor tissues, as well as lower ratio of ADH1B expression in tumor to non-tumor tissues, predict a shorter survival time after surgery. While, patients with lower ratio of ADH1B or ALDH2 expressions in tumor to non-tumor tissues had shorter recurrence-free survival time when compared to patients with higher ratio ($p = 0.002$, $p = 0.007$). Moreover, lower ADH1 and ALDH2 gene expression levels were statistically associated with bigger tumor size or higher AFP value.

Conclusions: In male PLC patients, lower ADH1 and ALDH2 expression levels associated with poorer overall survival and recurrence-free survival after surgery. Such prognostic predicting value was partly supported by the significant associations between lower ADH1 and ALDH2 expression levels and larger tumor size or higher AFP value.

O-220

Hepatitis E and ACLF: Predictors of mortality in Asia Pacific Region based on the AARC Data

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Acute hepatitis E (HEV) infection is a distinctive cause of ACLF in Asia-Pacific. However, most of available data has small sample size or are single center based experiences. We analyzed the APASL-ACLF research consortium (AARC) database to determine factors predictive of 90 day mortality in patients with ACLF triggered by acute HEV infection.

Materials and Methods: AARC consists of 24 tertiary centers across Asia-Pacific and maintains an online database for patients diagnosed to have ACLF according to APASL criteria. All patients who had ACLF with acute HEV were reviewed for the current study.

Results: 208 patients with HEV induced ACLF were analyzed. Mean age was 44.6 years and 85 % were male. Common causes of underlying chronic liver disease were alcohol (26.4 %), cryptogenic cirrhosis (26.4 %) and HBV infection (23.6 %). Overall, 59.4 % of patients survived and only one patient had liver transplantation. On univariate analysis, presence of the following at presentation was associated with mortality: jaundice, PSE, HRS, SBP, elevated leucocyte count, decreased platelets, elevated serum creatinine, high total bilirubin and INR. However on multivariate analysis, presence of PSE (5.84, 95 % CI 1.78–19.18, $p = 0.004$), HRS (4.35, 95 % CI 1.32–14.29, $p = 0.01$), total bilirubin at base line (1.05, 95 % CI 1.02–1.11, $p = 0.04$) were associated with mortality.

Conclusion: Acute HEV is a leading cause of ACLF in Asia Pacific and associated with high mortality. Presence of PSE, HRS and total bilirubin at base line were associated with mortality. Disease specific models to predict both survival and mortality are needed.

O-221

Usefulness of IgA anti-HEV antibody for diagnosis of acute HEV infection

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IgA class anti-hepatitis E virus antibody (IgA-HEV-Ab) is commonly used for screening of acute HEV infection in Japan, which is, however, no more than detection of antibody, namely indirect method. We evaluated the usefulness of IgA-HEV-Ab in patients of acute hepatitis due to HEV infection (AH-E). The diagnosis of AH-E was made by positivity of HEV-RNA in serum collected at early phase of liver disease. We determined IgA, IgM and IgG class of HEV-Ab and HEV-RNA in 49 patients of acute liver injury including 12 patients of AH-E. Among the patients of AH-E, one patient was serologically positive only for IgA-HEV-Ab and negative for the other antibodies. Another was negative for IgA-HEV-Ab and positive for the others. Residual 10 patients were positive for all classes of HEV-Ab. Among the remaining 37 patients whose serums were negative for HEV-RNA, one patient was positive for IgA-HEV-Ab. The sensitivity and specificity of IgA-HEV-Ab for the diagnosis of AH-E was 91.7 and 97.3 %, respectively. In two patients of AH-E undergoing immunosuppressive drugs for chronic rheumatoid arthritis, increase in IgA-HEV-Ab during the early phase was insidious compared to the others, and one of them showed negative result for IgA-HEV-Ab at the first exam as previously mentioned. In all AH-E cases, HEV genotype 3 was detected and HEV-RNA finally became negative. IgA-HEV-Ab is useful for diagnosis of AH-E, although its production may be affected by immunosuppressants and thus we should carefully evaluate the results in patients on such drugs.

O-222

TLR2 + monocyte and PD-1 + CD8 + T cell correlate with virologic breakthrough after switching to PegIFN

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Objective: The study was to characterize the immunological features responsible for virological breakthrough in 36 patients with chronic hepatitis B (CHB) treated with peginterferon (Peg IFN) alfa-2a after switching from entecavir (ETV) therapy (OSST trial, NCT00940485).

Methods: Toll-like receptors (TLRs) and programmed death 1 (PD-1) were evaluated dynamically by flow cytometry.

Results: Virological breakthrough was observed in 9 patients. Compared with the patients without virological breakthrough, the patients with virological breakthrough exhibited a lower rate of respond ($P = 0.046$), a lower rate of HBV DNA negativity ($P = 0.000$), as well as a higher level of HBsAg at week 48 ($P = 0.021$) and a smaller decline ($P = 0.012$) in HBsAg from baseline to week 48. Compared with the patients without virological breakthrough, the expression of TLR2 on CD14 + monocytes was significant lower in those with virological breakthrough ($P = 0.033$) at week 12. Moreover, patients

with virological breakthrough showed a significant increase of PD-1 expression on CD8 T cells from week 12 to week 24 ($P = 0.0014$). Interestingly, the patients with both TLR2 expression $<18\%$ at week 12 and PD-1 expression increasing $>22.5\%$ from week 12 to week 24 had a significantly higher rate of virological breakthrough than the patients who met either of conditions mentioned above (83.33 vs. 9.09 %, $P = 0.002$), and the patients who failed to meet any condition (83.33 vs. 11.11 %, $P = 0.005$).

Conclusions: Downregulation of TLR2 on CD14 monocytes and upregulation of PD-1 on CD8 + T cells correlate with virological breakthrough during the treatment with Peg IFN alfa-2a after switching them from ETV therapy.

O-223

Human liver CD8 + T cells have a tissue resident phenotype and differ from blood CD8 + T cells

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Background: The presence of a resident population of memory T cells (TRM) has been documented in many different tissues. Thus far, this population has been ill defined in the human liver. In this study we extensively phenotyped CD8 + T cells residing in the human liver as well as in paired blood in patients undergoing liver resection.

Methods: Hepatic lymphocytes and paired peripheral blood mononuclear cells were isolated from 7 patients undergoing liver resection. Phenotypic analyses on liver and peripheral blood CD8 + T cells were performed by flowcytometry. Localization of liver T cells was studied by immunohistochemistry. Functional capacity was analysed by qPCR after sorting of liver CD8 + T cells.

Results: In control patients, the majority of liver CD8 + T cells expressed CD69 (68 %) and a small part expressed CD103 (12.4 %). As CD69 positive CD8 + T cells expressed low levels of KLF2, these cells are likely to be TRM. Compared to peripheral blood, liver CD69 + TRM expressed high levels of CXCR6, and were marginally more activated, while they contained significantly less cytotoxic proteins.

Conclusions: In the liver, a specific CD8 + T cell population is present which has the phenotypic properties of TRM.

O-224

Impact of IL28B polymorphism on HCV viremia in dual HBV and HCV infected patients

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Background: Dual infection with HBV and HCV is not uncommon in HBV endemic region. Generally, one of the HCV or HBV be dominant while the other appeared undetectable or very low level of

viremia. The impact of IL-28B polymorphism on whether HBV or HCV viremia be dominant is unclear. The aim is to investigate the role of IL-28B genotype in dual HBV and HCV infected patients.

Material and method: Dual HBV-HCV infected patients whose HBV DNA and HCV RNA were both measured at treatment naïve status were recruited. HBV dominant defined as HBV DNA >2000 IU/mL while HCV dominant defined as HCV RNA detectable. HBV DNA and HCV RNA were measured by Roche Cobas Amplicor (limit of detection: HBV: 20 IU/ml; HCV: 43 IU/mL). IL28B genotyping (rs12979860 C > T, rs8099917 > G) were diagnosed by validated pyrosequencingTM assays.

Result: Nighty-five patients with HBV DNA and HCV RNA measured were recruited. HCV RNA presented positive in 87 (91.6 %) patients while HBV DNA >2000 IU/mL were found in 24 (25.3 %) patients. Both HCV RNA detectable and HBV DNA >2000 IU/mL was found in 22 (23.2 %) patients. Comparing age, gender, ALT level, cirrhosis, IL28B genotype between those with v s without HCV viremia, HBV DNA >2000 IU/mL v s ≤ 2000 IU/mL, respectively, rs12979860 CC genotype correlated with HCV viremia [89.7 % in HCV(+) v s 62.5 % in HCV(-), $P = 0.027$] while younger age correlated with HBV DNA >2000 IU/mL ($P = 0.05$).

Conclusion: Dual HBV-HCV infected patients with IL28B (rs12979860) CC allele have more HCV viremia rate but no impact on HBV viremia (>2000 IU/mL).

O-225

Acute Liver Failure due to HBV Reactivation via Immunosuppressive or Anticancer Therapies in Japan

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Aims: To clarify the clinical features of acute liver failure (ALF) and late onset hepatic failure (LOHF) due to HBV reactivation during and after immunosuppressive and/or anticancer therapies, we analysed data from nationwide survey of ALF in Japan.

Methods: A total of 1061 patients with ALF and LOHF, seen between 2010 and 2013, were enrolled from 742 hospitals. All patients showed a PT-INR of 1.5 or more within 8 weeks after the onset of disease symptoms.

Results: Among the total of 225 patients (24 %) who were diagnosed as having ALF or LOHF due to HBV infection, 90 patients (40 %) were HBV carriers. HBV reactivation due to immunosuppressive and/or anticancer therapies was responsible for the development of ALF in 50 carriers (56 %); they consisted of 26 patients positive for HBs-antigen and 24 patients negative for HBs-antigen (positive for anti-HBc and/or anti-HBs, before the onset of liver injuries). The outcome of carriers with ALF or LOHF showing HBV reactivation during and after immunosuppressive and/or anticancer therapies was unfavorable especially in those with resolved HBV infection (de novo hepatitis B); 22 (92 %) died without liver transplantation, while 1 survived without liver transplantation and 1 received liver transplantation.

Conclusion: HBV reactivation due to immunosuppressive and/or anticancer therapies was responsible for the development of liver injuries in more than half of HBV carriers with ALF and LOHF in Japan. National strategic plan for the prevention of HBV reactivation in patients receiving such therapies should be reconsidered to reduce the number of patients developing ALF and LOHF.

O-226

Compromised SDF1 production by liver stromal Cells suppress hepatocyte self replication in ACLF**Smriti Shubham, Dhananjay Kumar, Sheetalnath Rooge, Adil Bhat, Rakhi Maiwall, Archana Rastogi, Chhagan Bihari, Viniyendra Pamecha, Anupam Kumar, Shiv K Sarin**

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Background and aims: Acute insult triggers hepatic regeneration in both normal and chronic liver disease. Regenerative response is variable in ALF and ACLF. Regeneration in ALF mainly occurs through hepatocyte self replication. In ACLF, regeneration occurs mainly via activation and differentiation of hepatic progenitor cells. Underlying mechanisms for compromised hepatocyte proliferation in ACLF is not known. We investigated the role of hepatic microenvironment in defective hepatocyte proliferation in ACLF.

Patients and Methods: Liver biopsy/explant and peripheral/hepatic vein blood was collected from ACLF (n = 33) and ALF (n = 21) patients. Plasma growth factors were analysed by Cytokine array. Growth factors levels in tissue was confirmed by RT-PCR. Immunohistochemistry was performed for CXCR7 and Ki67.

Results: ACLF plasma showed more than twofold decrease in AFP, HGF, LIF, M-CSF, SDF and increase in EGF and GM-CSF in comparison to ALF. Ki67 significantly correlated with serum HGF (R = 0.537, p < 0.001) and SDF1 (R = 0.369, p = 0.006) levels in both ALF and ACLF. HGF and SDF1 protein level in serum (p < 0.0002, p = 0.002) and mRNA levels (p = 0.002, p = 0.0155) in tissue were significantly decreased in ACLF as compared to ALF suggesting that decreased HGF and SDF1 might be responsible for poor hepatocyte replication in ACLF. RT-PCR analysis showed significantly reduced CXCR7, Id1 and Wnt2a mRNA levels in ACLF liver tissue in comparison to ALF. IHC shows decreased CXCR7 positive liver endothelial cells in ACLF. Data suggests decreased liver SDF1 might be responsible for defective CXCR7-Id1-HGF/Wnt2a signaling, leading to compromised hepatocyte proliferation in ACLF.

Conclusion: Significantly lower SDF1 level might be responsible for decreased HGF production and subsequent compromised hepatocyte proliferation in Acute-on-chronic liver failure.

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Natural history and outcome of stage 3 AKI in patients with ACLF: a multinational cohort study**Rakhi Maiwall¹, Mamun A Mahtab², Yogesh K. Chawla³, Soek S. Tan⁴, Harshad Devarbhavi⁵, Saeed S. Hamid⁶, C. E. Eapen⁷, Hasmik Ghazinyan⁸, Zhongping Duan⁹, Ajit Sood¹⁰, Guan H. LEE¹¹, A. K. Dokmeci¹², Laurentius A. Lesmana¹³, Diana A. Payawal¹⁴, Ashok K. Choudhury¹⁵, Manoj K. Sharma¹⁶, Priyanka Jain¹⁷, Suman L. Nayak¹⁸, Shiv K. Sarin¹⁹, AARC Group²⁰**¹Institute of Liver and Biliary Sciences, New Delhi, India;²Bangabandhu Sheikh Mujib Medical university, Dhaka, Bangladesh;³PGIMER, Chandigarh, India; ⁴Selayang Hospital University ofMalaya, Kuala Lumpur, Malaysia; ⁵St John medical college,Bangalore, India; ⁶Aga Khan university hospital, Karachi, Pakistan;⁷C M C Vellore, India; ⁸Nork Clinical Hospital of InfectiousDiseases, Yerevan, Armenia; ⁹Beijing Youan Hospital, CapitalMedical University, Beijing, China; ¹⁰CMC, Ludhiana, India; ¹¹Yong

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Background and Aims: Currently, there are no studies investigating the natural history of stage 3 AKI (AKI-3) defined as increase in serum creatinine (sCr) >3 fold or >4 mg/dl or requirement of renal replacement therapy (RRT) in patients with ACLF.

Patients and Methods: We specifically evaluated AKI-3 in a large prospective multicentric-multinational cohort of ACLF patients.

Results: Of 2063 patients, 83.6 % males, aged 43 ± 12.8 years, 139 (23.8 %) patients had AKI-3 at admission; 132 (95 %) with sCr > 4 mg/dl; 7 (5 %) requiring RRT while 146 (26.7 %) had progression to AKI-3 during hospital stay; increase in sCr > 3-fold in 43(29 %), sCr > 4 mg/dl in 68 (47 %) and 35 (24 %) required RRT. Mean MELD and SOFA scores were 40 ± 8.5 and 12.5 ± 3 respectively and 217 patients (76 %) died on follow up. There was no significant difference in mortality in patients who had AKI-3 at admission versus those who developed on follow up (Log rank p = 0.8) and between different subcategories of AKI-3 (p > 0.05). High MELD (p = 0.01, HR 1.08, 95 % CI 1.02–1.15) and serum lactate (p = 0.003, HR 1.4, 95 % CI 1.12–1.76) were independent predictors of mortality in these patients. Of patients who required RRT, (SLED-54, CRRT-7) only 13 % survived 1-month. In these patients, serum potassium (>5) (p = 0.037, OR 5.37, 95 % CI 1.11–26.1), sCr (>8.8) (p = 0.04, OR 2.44, 95 % CI 1.02–5.83) and SOFA score (<14) (p = 0.23, OR 13.1, 95 % CI 1.32–129.6) were independent predictors of survival.

Conclusion: AKI-3 is seen in almost 50 % of patients with ACLF and associated with very high mortality. RRT is a futile exercise in patients with ACLF when done in patients without hyperkalemia, low sCr and high SOFA scores.

O-228

Clinical analysis of AKI in patients with HBV-related ACLF**Xin-xing Shi, Peng Zhu, Guo-hua Yan, Chen Liu, Chang-Jiang Zhang, Yu-Ming Wang**

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Objective: This study analyzes the clinical characteristics and long term prognosis of HBV related ACLF patients with accompanying Acute kidney injury (AKI).

Methods: 1167 HBV related ACLF patients hospitalized from January 2010 to January 2015 were collected retrospectively, who were divided into AKI group (n = 308) and non-AKI group (n = 859). Patients were followed up to observe clinical characteristics and to analyze long term overall survival (OS) and associated risk factors.

Results: Incidence of AKI was 26.4 % in ACLF patients. TBIL, INR, sCr and MELD score were higher for AKI group. Patients in the AKI and non-AKI groups had 30 day OS of 44.8 and 70.3 %; 90 day OS of 17.9 and 55.4 %; and 1 year OS of 15.6 and 51.2 %, respectively, showing significant differences in all three parameters (P < 0.001). Significant differences were observed between different AKI stage subgroups. Incidences of ascites, SBP, upper gastrointestinal bleeding, infection and HE were higher for the AKI group (P < 0.001). High WBC, ALT and MELD score were risk factors for 30 day

mortality, whereas hepatic encephalopathy, high MELD score and low PLT were risk factors for 90 day mortality. KDIGO and AKIN criteria showed high agreement in diagnosing and staging AKI in HBV related ACLF patients ($K = 0.807$, $P < 0.001$).

Conclusion: AKI is associated with poor outcome, particularly with increased short term mortality, in ACLF patients. Both KDIGO and AKIN criteria can be used for staging of AKI in HBV-related ACLF patients.

O-229

Effective early interventions are essential to attain high survival rate

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We believe that the treatment of acute liver failure comprises three parts as follows: provision of ALS, treatment of underlying liver disease/s, and prediction of development of fulminant hepatic failure. Initially, we developed an ALS system. Almost all patients with acute liver failure regained consciousness; however, this treatment improved the survival rate of only those patients with acute type. In patients with subacute type, destruction of the liver cells continues even after the appearance of hepatic encephalopathy. Therefore, we recognized the necessity of treating underlying liver diseases. After diagnosis of the underlying liver disease, we found that a substantial proportion of subacute type had impaired liver regeneration. Evaluation of the risk of acute liver failure at the stage of acute hepatitis and prevention of liver cell destruction are crucial to the achievement of higher survival rates in patients with subacute liver failure. We developed a prediction formula that can evaluate risk of developing acute liver failure. The first treatment goal must be to stop the destruction of liver cells. We deem the ALS treatment to be indispensable for controlling the fatal symptoms of acute liver failure. Considering the shortage of organ donors, early medical intervention is very important. After establishment of the treatment system, we achieved about 70 % of survival rate in patients with acute liver failure. We conclude that effective early interventions are essential for improving survival rates among patients with acute liver failure.

O-230

Risk factors for liver failure from immunosuppressive therapy-induced hepatitis B virus

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Objective: Hepatitis B virus reactivation (HBVr) is a common seen complication of immunosuppressive therapy. This study describes the risk factors of HBVr-related liver failure.

Methods: From 2001 to 2013, all 195 HBVr patients undergoing immunosuppressive therapy in Southwest Hospital were included in this retrospective study.

Results: This study revealed that the strongest protective factor against liver failure was NAs treatment before immunosuppressive therapy [odds ratio (OR), 0.112; 95 % confidence interval (CI), 0.017–0.715, $P = 0.021$]. The strongest predictor of liver failure was liver cirrhosis (OR, 69.659; 95 % CI 12.526–387.394, $P = 0.000$),

and this was followed by alcohol consumption (OR, 4.511; 95 % CI 1.25–16.165, $P = 0.021$), HBV DNA levels after immunosuppressive therapy (OR, 1.989; 95 % CI 1.423–2.78, $P = 0.000$), age (OR, 1.065; 95 % CI 1.021–1.112, $P = 0.004$), and peak ALT value after HBVr (OR, 1.002; 95 % CI, 1.001–1.004, $P = 0.000$). The cutoff values of HBV DNA levels after immunosuppressive therapy, peak ALT values after immunosuppressive therapy, and age determined to predict liver failure were 6.09 log₁₀, 122, and 45.5, respectively. There was a significant in overall failure rate between groups receiving rituximab-containing chemotherapy and chemotherapy without rituximab.

Conclusion: This study indicates the need for continued surveillance of risk factors including non-NA treatment prior to immunosuppressive therapy, alcohol consumption, liver cirrhosis at baseline, HBV DNA level after immunosuppressive therapy, older age, and peak ALT value after HBVr in HBVr patients to prevent progression of HBVr-hepatitis to liver failure.

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A predictive formula for the prognosis of patients with acute-on chronic liver failure

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Background & aim: The prognosis of acute-on chronic liver failure (ACLF) is extremely poor. We aimed to establish methods for its severity to identify the patients with a poor prognosis.

Methods: The laboratory data at admission of 30 ACLF were evaluated. Three established prognosis prediction models (model for end stage liver disease [MELD]; MELD modified by serum sodium concentration, [MELD-Na]; and the Japan hepatic encephalopathy prediction model [JHEPM]) were assessed using area under the receiver operating characteristic curve (AUROC) values.

Results: J-HEPM was able to predict the outcome of the ACLF subjects (AUROC, 0.93) although MELD and MELD-Na presented lower predictive value (AUROC; 0.438 or 0.439, respectively). The high MELD-Na or MELD scores in the deceased patients correlated with the PT-INR value ($r = 0.837$ or $r = 0.719$, respectively), while the high scores in the surviving patients correlated with serum creatinine level ($r = 0.859$ or $r = 0.849$, respectively).

Conclusions: The JHEPM effectively predicted the prognosis of liver failure in patients with ACLF.

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Risk and adverse outcomes of fractures in patients with liver cirrhosis: two nationwide studies

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Background: Falls were identified as a complication for people with liver cirrhosis (LC). This study evaluated fracture risk and post-fracture outcomes in patients with LC.

Methods: We identified 3941 adults aged 20 years and older newly diagnosed with LC using the Taiwan National Health Insurance Research Database from 2000 to 2003. Comparison cohort consisted of 15,764 adults without LC randomly selected by frequency matching in age and sex. Followed-up events of fracture from 2000 until 2008 were ascertained from medical claims. Adjusted hazard ratios (HRs) and 95 % confidence intervals (CIs) of fracture associated with LC were calculated in the multiple Cox proportional hazard models. Another nested cohort study of 600,828 hospitalized fracture patients analyzed for adjusted odds ratios (ORs) and 95 % CIs of adverse events after fracture among patients with and without LC between 2006 and 2013.

Results: The incidences of fracture for people with and without LC were 28.0 and 16.9 per 1000 person-years, respectively. Compared with control, the adjusted HR of fracture was 1.71 (95 % CI 1.55–1.87) for LC patients. Previous LC was associated with risks of septicemia (OR 1.87, 95 % CI 1.68–2.0), acute renal failure (OR 1.77, 95 % CI 1.43–2.18), and mortality (OR 1.71, 95 % CI 1.45–2.01) after fracture.

Conclusion: LC was associated with higher risk of fracture. Patients with LC had more complications and mortality after fracture. Fracture prevention and attention to post-fracture adverse events are needed for this susceptible population.

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Non invasive APRI Score in Cirrhosis correlates with invasive Hepatic venous pressure gradient?

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Background: Hepatic Venous Pressure Gradient (HVPG) is the only recommended means to measure portal hypertension and prognosticate cirrhosis. APRI is simple noninvasive marker of hepatic fibrosis. Aim-Compare non invasive APRI score with invasive HVPG for portal hypertension and determine usefulness of APRI in portal hypertension.

Methods: Study included patients of cirrhosis between ages 18–70 years.

Results: Study included 147 patients with median age 52.39 ± 10 years; 120 (81.6 %) males. Etiology of cirrhosis were alcohol 69 (47 %), viral 20 (13 %), cryptogenic 46 (31 %) and others 12 (8 %). Mean CTP score and mean MELD score were 7.69 ± 1.91 and 13.29 ± 4.90 respectively. Median HVPG was 17.90 ± 4.74 mmHg. Maximum Youden's index was 0.4676 which corresponded to a cut-off value of 0.946 of APRI. The ROC curve to study the performance of APRI for predicting portal pressure (HVPG >12 mmHg) had area under curve 0.712 ($P = 0.001$). APRI of 0.946 had a sensitivity 71.76 %, specificity 75 %, positive predictive value 95.92 %, negative predictive value 24.49 %, and diagnostic accuracy 72.10 % for predicting HVPG >12 mmHg. There was significant difference (Spearman's Rho = 0.370; $p \leq 0.001$) in median APRI of patient with HVPG ≤ 12 mmHg [APRI-0.83 (0.32–3.33)] and those with HVPG >12 mmHg [APRI-1.40 (0.29–12.22)] respectively.

Conclusion: APRI score of 0.946 seems to have an acceptable accuracy for prediction of high portal pressure. APRI is a simple, non invasive and cost-effective parameter for diagnosis of high portal pressure in patients with cirrhosis.

Keywords: Cirrhosis, HVPG, APRI, Portal hypertension.

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The beneficial effects of curcumin in cirrhotic rats with portal hypertension

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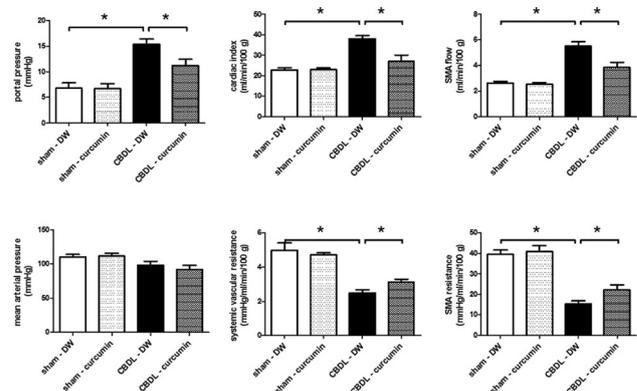
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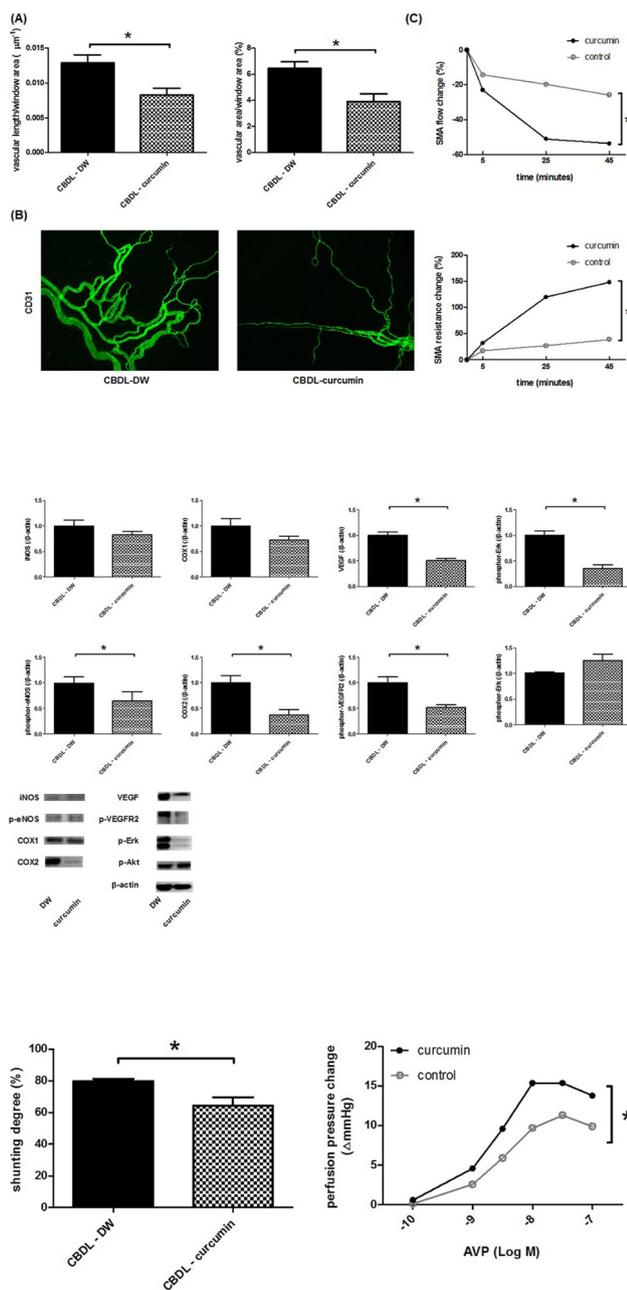
Background: In liver cirrhosis with portal hypertension, the derangement of vasoactive substances bioavailability leads to increased intrahepatic vascular resistance and splanchnic vasodilatation. In recent years, angiogenesis has also been implicated in the development of increased portal inflow and collaterals. Curcumin, a traditional seasoning, has aroused much attention for its vasoactive and anti-angiogenesis actions. Whether it is beneficial in the control of portal hypertension has not been explored.

Methods: Liver cirrhosis was induced by common bile duct ligation (BDL) in Sprague-Dawley rats. Sham-operated rats were controls. BDL and sham rats were randomly allocated to receive curcumin (600 mg/kg per day) or vehicle since the 15th day after BDL. On the 29th day, portal hypertension-related parameters were surveyed. Portosystemic collateral in situ perfusion was performed to evaluate vascular activity.

Results: Chronic curcumin treatment decreased portal pressure, cardiac index and increased systemic vascular resistance in cirrhotic rats. In splanchnic system, curcumin decreased superior mesenteric artery (SMA) flow and increased SMA resistance. Mesenteric abnormal angiogenesis was attenuated by curcumin. Acute administration of curcumin significantly induced splanchnic vasoconstriction. The mesenteric proteins expression of phosphor-eNOS, COX2, VEGF, phosphor-VEGFR2 and phosphor-Erk decreased at the same time. In collateral system, curcumin decreased portosystemic shunting and induced collateral vasoconstriction.

Conclusions: Chronic treatment of curcumin in cirrhotic rats ameliorated portal hypertension and portosystemic collaterals. Curcumin attenuated splanchnic hyperdynamic circulation by inducing vasoconstriction through inhibition of phosphor-eNOS and by decreasing mesenteric angiogenesis via VEGF pathway blockade





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Role of portal flow dynamics in the hepatic morphological changes associated with liver fibrosis

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Human liver uniquely changes its shape with advance in fibrosis. We have reported a significant role of regional portal blood flow in liver regeneration after resection (Dig Dis Sci, 2013).

Aim: The aim of this study was to investigate possible role of portal flow change in the unique morphological changes.

Objects/Methods: Six patients with liver diseases were analyzed. According to MD-CT, 3D structure of portal branches was constructed, and mesh models of portal branches were created. Blood flow in each portal branch was simulated employing computational flow dynamics (CFD) software (total flow: 1200 ml/min, output pressure: 0 Pa). To simulate the decreased portal blood flow in fibrotic livers, the flow rate was reduced to 800 ml/min or 600 ml/min. The distribution of flow in each portal branch was compared.

Results: When the portal flow rate was reduced from 1200 to 600 ml/min, the distribution of flow in each portal branch non-uniformly changed. Rates of the distribution change were between -25.8 % and +10.6 %. Intriguingly, the flow distribution significantly decreased in Segments 6 and 4, where hepatic atrophy is often observed in human cirrhosis. Meanwhile, the distribution significantly increased in Segments 2 and 8, where compensated hypertrophy is ordinarily detected. The flow distribution in portal branches with low flow rate at 1200 ml/min greatly decreased at 600 ml/min.

Discussion: Change in the distribution of intrahepatic portal flow associated with the decreased porta flow in cirrhosis is possibly heterogeneous. The continuous non-uniform change in intrahepatic flow distribution is possibly associated with the unique morphological change in cirrhotic livers.

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Hemodynamic response to propranolol for treatment of portal hypertension in a center in Singapore

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Background/Aim: Propranolol reduces the risk of acute variceal bleeding (AVB) in cirrhotic patients with portal hypertension. However, satisfactory hemodynamic response is only achieved in 30–60 % of patients treated with propranolol. We aim to study the rate of hemodynamic response in portal hypertensive patients treated with propranolol in our center and to compare incidence of AVB between responders and non-responders.

Methods: Retrospective review of 69 patients with portal hypertension-related varices who underwent hepatic venous pressure gradient (HVPG) measurement after optimization of propranolol dose (to a resting heart rate 55–60 and/or systolic BP 90–100 mmHg). Satisfactory hemodynamic response was defined as a HVPG <12 mmHg or ≥20 % reduction from baseline. Clinical features and incidence of AVB were compared between responders and non-responders.

Results: Mean age was 56 ± 10 years, with 55 % males. 28 patients (41 %) achieved satisfactory hemodynamic response to propranolol. Median dose of propranolol was 90 mg/day (range 10–240). There were no significant differences in the clinical features or mean propranolol dose between responders and non-responders. Mean HVPG of responders was 8.4 ± 3.7 mmHg compared to 19.5 ± 5.9 mmHg in non-responders (p < 0.001). In patients with repeat HVPG, responders had mean reduction of -3.9 ± 4.0 mmHg compared to an increase of 3.1 ± 4.5 mmHg in non-responders (p = 0.004). Incidence of AVB was lower in responders (1/28, 3.6 %) compared to non-responders (7/41, 17.1 %), but was not statistically significant (p = 0.08).

Conclusion: Despite an optimized dose of propranolol, only 41 % of patients with portal hypertension achieved the desired hemodynamic response, which is associated with a lower risk of AVB. This

highlights the importance of HVPG measurement in the management of portal hypertension.

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Clinical characteristics and risk factors of portal vein thrombosis in liver cirrhosis

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Background: Portal vein thrombosis (PVT), an common complication in the patients with cirrhosis, is often neglected and influenced on clinical manifestation and prognosis for cirrhosis patients.

Objective: To investigate the clinical characteristics and risk factors of portal vein thrombosis (PVT) in patients with cirrhosis.

Methods: 65 cirrhosis patients with PVT as PVT group and 70 without PVT as control group admitted in our hospital from 2013 to 2015 were collected, and their clinical data as well as risk factor for PVT were retrospectively analyzed. General information, laboratory results, imaging findings, clinical manifestations and complications were recorded and analyzed. Clinical characteristics were compared, and corresponding risk factors were selected.

Results: The results of age, sex, nation, etiology, white blood cell, platelet, INR, APTT, fibrinogen, creatinine, total bilirubin, splenic vein diameter, diarrhea, intestinal obstruction, hepatorenal syndrome and hepatic encephalopathy in two groups were no statistically differences ($p > 0.05$). Compared with Non-PVT group, the differences in D-dimmer, FDP, hemoglobin, albumin, portal vein diameter, spleen thickness, spleen length, Child-Pugh score, splenectomy, and clinical manifestation including abdominal pain, fever, upper gastrointestinal bleeding and spontaneous bacterial peritonitis in PVT group were statistically significant ($P < 0.05$). D-dimmer (OR = 4.290, $P < 0.000$) and portal vein diameter (OR = 1.294, $P = 0.023$) were independent risk factors for the cirrhosis patients with PVT.

Conclusion: The incidence of PVT is high in cirrhosis patients with high level of D-dimmer and FDP, wider diameter of portal vein, splenomegaly and splenectomy. Clinical complications is significantly increased in cirrhosis patients with PVT.

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The effectiveness of danaparoid sodium for portal vein thrombosis

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Background: Danaparoid sodium has been reported to be an effective anticoagulant agent for portal vein thrombosis (PVT). In this study, we assessed the effectiveness of danaparoid sodium for PVT.

Methods: The subjects were 30 patients (M/F = 18/12, mean age 64.5 years) who were diagnosed PVT and were treated with dana-

paroid sodium at our institute from April 2006 to December 2014. 1250 U/body of danaparoid sodium was administered twice a day intravenously for 14 days. Enhanced Computed Tomography (CT) was taken to evaluate PVT. We assessed if some factors had correlation with treatment effect by statistical analysis.

Results: Of 30 patients, 18 patients (60 %) had neoplasm and 13 patients (43 %) had liver cirrhosis or portal hypertension. PVT appeared in 3 patients (10 %) after laparotomy, in one patient (3 %) after transcatheter arterial chemoembolization (TACE), and in one patient (3 %) after radiofrequency ablation for hepatocellular carcinoma. In 29 patients, treatment effect was evaluated by CT images after treatment. In 24 of 29 patients (80 %), PVT disappeared or was markedly reduced in size. In 5 patients (17 %), the treatment was ineffective. There was no significant correlation between treatment effect and the time from diagnosis to treatment. Plasma AT-III level was measured in 12 of 19 patients before treatment. Mean activity of plasma AT-III was 68.3 %. In 7 patients, plasma AT-III level was lower than the standard value (80–120 %). However, in all of 12 patients, most of the PVT disappeared.

Conclusion: Danaparoid sodium was effective for PVT.

O-239

Clinical characteristics at the time of start of diuretics for ascites predict their survival

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Background and Aims: Ascites is a common complication as the degree of liver injury and fibrosis proceeds. The first-line treatment is recommended to be sodium restriction and the use of diuretics. However, the clinical courses after the first diuretics administration vary greatly. Here, we conducted this retrospective analysis to identify the influential factors for survival in patients with ascites due to cirrhosis.

Methods: 64 patients with decompensated liver cirrhosis and ascites were retrospectively enrolled and analyzed. The analysis started from the time as first diuretics were introduced. We stratified them into two groups, those who survived more than 2 years after starting diuretics (survival group), and others who died within two years (non-survival group). Clinical characteristics were compared between the groups and analyzed.

Results: Forty patients were classified as survival while the others were classified as non-survival. Patients in non-survival group showed significantly higher total bilirubin, serum sodium concentration, PT-INR, the incidence of hepatic encephalopathy, and significantly lower serum albumin levels. There was no significant difference in transaminase, hemoglobin, platelet count and incidence of other complications. They showed significant difference in Child-Pugh and MELD score, but not in AST/ALT ratio, FIB-4 index and APRI.

Conclusion: Between two groups, total bilirubin, PT-INR, albumin, hepatic encephalopathy showed significant differences. These factors, as well as ascites, are evaluated in the Child-Pugh scoring system, therefore, Child-Pugh score maybe useful to predict survival, too. Hyponatremia means loss of the balance of fluid volume, and might be a sign of progressive of renal or hepatic dysfunction.

O-240

The true efficacy of tolvaptan in patients with refractory ascites

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Backgrounds and Aims: We conducted this study to clarify the true outcomes of TLV in patients with refractory ascites compared to historical controls.

Patients and Methods: In this retrospective study, we enrolled 80 refractory ascites patients treated with TLV and extracted another 80 refractory ascites patients treated with large volume paracentesis as control group. The patient's backgrounds and laboratory data including liver function were not matched, because of small number of patients. We compared the patient's backgrounds, laboratory data, treatment outcomes, and cumulative incidence rates between the two groups. We also elucidated factors which contributed to these incidences using Cox hazard proportional multivariate analysis. Incidences were defined as an additional invasive procedure including paracentesis, or need for hospitalization for any reason.

Results: Serum sodium level was significantly lower in TLV group (133 vs. 136 mEq/l, $P = 0.02$). However, there were no significant differences about other parameters between the two groups. Cumulative incidence rate was significantly higher in the control group: median time for incidence was 33 days in TLV group and 14 days in the controls ($P = 0.01$). Cox hazard proportional multivariate analysis indicated that use of TLV (OR: 0.52 $P = 0.01$), uncontrollable hepatocellular carcinoma (OR: 2.03, $P = 0.01$), and higher sodium level (OR: 0.97 per 1 mEq/l, $P = 0.04$) as independent factors which contributed to incidence.

Conclusions: Administration of TLV achieved not only better control of refractory ascites but also better QOL avoiding additional invasive procedure including paracentesis or need for hospitalization compared to conventional ascites treatments.

O-241

Predictive value of the efficacy of tolvaptan in patients with refractory ascites in liver cirrhosis

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Background/Aims: Recently, the efficacy of the tolvaptan, vasopressin V2 receptor antagonist, for the treatment of refractory ascites in liver cirrhosis has been reported. This study was to investigate clinical features and therapeutic effects with tolvaptan in patients with ascites in liver cirrhosis.

Methods: 50 patients with ascites in liver cirrhosis (hepatitis B, 1; hepatitis C, 22; alcoholism, 11; others, 16) were enrolled in this prospective study. After adding tolvaptan (3.75–7.5 mg/day) to conventional diuretics, various data were collected at baseline (day 1) and 1 week post-tolvaptan treatment (day 8) for all patients.

Results: 36.0 % of patients decreased over 2 kg of their body weight (responder) after the administration of tolvaptan. Tolvaptan was also effective against patients with liver failure and portal vein thrombosis.

Serum BUN, plasma renin, and plasma aldosterone of non-responder (decrease under 2 kg or increase of their body weight) compared with responder was significantly higher ($p < 0.05$). It is suggested that tolvaptan is not effective on the pathogenesis of underfilling. Receiver operating characteristics (ROC) analysis showed good prediction by BUN for responder (AUC = 0.77). And cutoff index of BUN was 26 mg/dL.

Conclusions: Tolvaptan is useful and safe for the treatment of refractory ascites in liver cirrhosis. This study presented that a clinical parameter of BUN before administration could predict the response of tolvaptan.

O-242

Tolvaptan improved refractory ascites and overall survival in patients with hyponatremia

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Backgrounds and Aims: Recently, some studies have reported the efficacy and safety of tolvaptan (TLV) for treating hyponatremia and ascites in patients with decompensated cirrhosis.

Patients and Methods: In this retrospective study, we enrolled 40 decompensated cirrhosis patients with hyponatremia and ascites using TLV between October 1st 2012 and December 31 2014. We defined the improvement of ascites as weight loss for more than 5.0 % and alteration of hyponatremia as serum sodium level over 135 mEq/L during the follow-up period. We divided the patients into two groups according to the improvement of hyponatremia and compared the laboratory data between the two groups. We also analyzed the factors which contributed to the improvement of ascites, hyponatremia, and overall survival using multivariate analysis.

Results: Of the 40 patients, 26 patients (65.0 %) were Child-Pugh class C and 23 patients (57.5 %) had advanced hepatocellular carcinoma (HCC: BCLC stage B, C, or D). Ascites had been improved in 16 patients (40.0 %). Hyponatremia had been improved in 19 patients (effective group) and not been improved in 21 patients (ineffective group). The cumulative survival rates significantly differed between the two groups ($P = 0.03$); 100, 78.9, and 62.2 % at 10, 30, and 60 days in effective group and 90.5, 47.6, and 32.7 % in ineffective group, respectively. Multivariate analysis showed that uncontrollable HCC (HR: 3.29, $P = 0.01$), and alteration of hyponatremia (HR: 0.24, $P = 0.01$) as independent factors which contributed to overall survival.

Conclusion: TLV improved not only ascites but also overall survival in decompensated cirrhosis patients with hyponatremia.

O-243

The prognosis of patients with liver cirrhosis and refractory ascites treated by Tolvaptan

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Tolvaptan is a new diuretic for cirrhotic patients with ascites which acts as a vasopressin receptor 2 antagonist. Recently several reports

showed the effect of Tolvaptan for refractory ascites; however, the prognosis of patients treated with Tolvaptan is unknown.

Methods: Tolvaptan treatment was started during hospitalization. We investigated 22 patients with liver cirrhosis and refractory ascites treated by Tolvaptan, who could be followed-up after hospital discharge. Body weight, urine volume, serum sodium/ammonia/creatinine levels before and after Tolvaptan treatment were measured. Survival rates at 3 and 6 months after the start of Tolvaptan treatment and hospital readmission rates were evaluated.

Results: Average age: 70.3 ± 12.2 years. Male/Female: 14/15. Patient background: HCV or HBV; 8, AIH; 1, PSC; 1, alcohol; 6, nonBnonC; 6. Child-Pugh score: A; 0, B; 11, C; 11. Body weight was significantly decreased to -2.5 ± 4.1 % at day 7 ($p = 0.001$) followed by Tolvaptan treatment, and -6.6 ± 6.1 % at the hospital discharge ($p < 0.001$). Urine volume was increased to 2.9-fold on the next day, and 2.4-fold at day 7 ($p < 0.001$) compared with before Tolvaptan treatment. No serious serum sodium, ammonia or creatinine increase was detected within 7 days after the start of Tolvaptan treatment. However, nine patients (40.9 %) were readmitted to the hospital within 30 days, mostly because of retention of ascites. Survival rates were 77.3 % (17/22) at 3 months and 45 % (11/20) at 6 months.

Conclusion: Tolvaptan treatment was effective during hospitalization: however, almost forty percent of patients were readmitted soon after hospital discharge and over half of patients died within 6 months. The management of ascites in outpatient care is important for these cirrhotic patients treated with Tolvaptan.

O-244

Stepwise combining fibroscan and routine markers improve detecting compensated CHB cirrhosis

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Aim: Significant inflammation may overestimate liver stiffness and result in false positive diagnosis by transient elastography for CHB cirrhosis detection. This study tries to further improve the performance of transient elastography by stepwise combination with routine biomarkers.

Methods: Total of 236 compensated CHB patients with alanine transferase lower than 5 times upper limit of normal, liver biopsies, transient elastography and routine blood tests were included. Performance of combining transient elastography and routine biomarkers were analyzed.

Results: The area under ROC for detecting cirrhosis was 0.876 for transient elastography, 0.794 for FIB-4, 0.785 for CDS, 0.765 for API, 0.715 for APRI and 0.661 for AAR, respectively. The area under ROC for significant fibrosis was 0.844, 0.662, 0.580, 0.595, 0.695 and 0.510 in the same order. The proportion of patients determined as cirrhosis or non-cirrhosis was 66.5 % by transient elastography, 41.1 % by FIB-4, 30.5 % by CDS, 14.4 % by API and 24.2 % by APRI, respectively; the number for significant fibrosis was 55.5 % by transient elastography, 11.9 % by APRI and none by the other serum markers. Stepwise combination of transient elastography and CDS, FIB-4 or APRI increased positive predictive value of confirming

cirrhosis diagnosis from 0.677 to 0.84, 0.808 and 0.724, respectively; and the proportion of patients being determined the state of cirrhosis and obviating liver biopsy was up to 77 %.

Conclusions: By transient elastography based stepwise combination with readily available serum markers, performance of detecting compensated CHB cirrhosis could be significantly improved in terms of diagnosis accuracy and proportion of obviating liver biopsy.

O-245

Liver cirrhosis and cancer: comparison of mortality and economic burden

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Aim: This study estimates the mortality and economic burden of liver cirrhosis and compares it with that of five major cancers (stomach, colon, liver, lung, and breast).

Methods: We used both the Cause of Death statistics and NHISCD which provides a cohort data of 1,025,340 representative sample in Korea. And, we used NHID to study economic burden across the diseases. Results: According to the NHISCD, 800 out of 2609 liver cirrhosis patients in 2002 died during the following eight years while 1555 out of 5288 major cancer patients died. The relative mortality of liver cirrhosis was greater than that of five major cancers [odds ratio = 1.56 (95 % CI 1.39–1.76)]. When liver cirrhosis was compared with four major cancers excluding liver cancer, the relative mortality was still greater for liver cirrhosis [odds ratio = 1.57 (95 % CI 1.39–1.78)]. We calculated death-related economic loss amounted to 1778 billion Won for liver cancer and 1452 billion Won for other liver disease. The respective numbers were 1261 for stomach, 968 for colon, 1727 for lung, and 593 billion Won for breast cancer.

Conclusions: The mortality of liver cirrhosis is greater than that of five major cancers. And the economic burden of liver disease is immense in comparison with major cancers because of the associated high mortality and morbidity, especially among the economically active population. This implies that we need to prioritize development of appropriate health interventions for liver cirrhosis.

O-246

Sleep disturbances in patients of liver cirrhosis with minimal hepatic encephalopathy

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Background: Minimal hepatic encephalopathy (MHE) represents the mildest form of hepatic encephalopathy without cognitive impairment, but patients have abnormal neuropsychologic and/or neurophysiologic findings indicative of cerebral dysfunction. Sleep disturbances and excessive daytime sleepiness have been reported in patients with cirrhosis of liver. In this study we compared various sleep parameters including Polysomnography (PSG), Epworth sleepiness scale (ESS), Pittsburg sleep quality index (PSQI) and Health related quality of life (HRQOL) in patients of liver cirrhosis with and without MHE.

Methods: 100 patients of cirrhosis (50 with MHE and 50 without MHE) enrolled. Assessment of MHE was done by psychometric hepatic encephalopathy score (PHES). Basic characters were similar in both the groups including Child and MELD score. PSG, ESS, PSQI and HRQOL using SF-36 performed in both the groups and compared. Statistical tests used as appropriate.

Results: Mean PHES score in patients with MHE was -7.64 as compared to -1.22 in patients without MHE. PSG parameters such as total sleeping time (261.4 ± 36.3 vs 322.6 ± 46.4 min), sleep efficiency (46.3 ± 11.2 vs 60.1 ± 12.6 %) and REM sleep (21.5 vs 28.4 %) were significantly lower whereas sleep latency (43.3 ± 6.6 vs 24.4 ± 5.3 min) was significantly higher in patients with MHE. Patients with MHE had poor sleep quality (PSQI 8.6 vs 4.7), excessive day time sleepiness (ESS 12.5 vs 8.3) and poor quality of life (SF-36 composite score 39.6 vs 61.4) with p value <0.05 .

Conclusions: MHE disturbs quality of sleep, causes excessive day time sleepiness, disturbs sleep pattern and impairs health related quality of life in patients with cirrhosis.

O-247

Influence of CYP2D6 and β 2-AR gene polymorphisms on hemodynamic response to propranolol in Chinese

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Background and aim: Propranolol is widely used to prevent gastroesophageal variceal bleeding; however, some patients could not benefit from propranolol. This study is to evaluate the relationship between CYP2D6 and β 2-adrenergic receptor (β 2-AR) gene polymorphisms and the hemodynamic response to propranolol in Chinese Han patients.

Methods: The clinical data of patients with gastroesophageal varices undergoing Hepatic venous pressure gradient (HVPG) measurement before and 7 days after oral propranolol administration in our department were collected. 4 single nucleotide polymorphisms of CYP2D6 and β 2-AR genes were detected. The relationship was identified by logistic regression model.

Results: 30 patients were involved in the analysis. 60 mg propranolol twice each day was well tolerated by all the patients. The initial and secondary average of HVPG was 17.4 ± 5.8 vs. 13.2 ± 4.8 mmHg

respectively ($t = 5.726$, $P < 0.001$). 21 patients responded to propranolol. The mean reduction value of HVPG was 6.6 ± 3.6 mmHg (range from 3 to 19). Genotype analysis showed: 20 homozygotes for C/C188 and 10 for heterozygous C/T188, 8 homozygotes for G/G4268 and 22 heterozygotes for G/C4268, 14 homozygotes for Gly16 and 10 heterozygotes and 6 homozygotes for Arg16, 27 homozygotes for Gln27 and 3 heterozygotes. The multivariate logistic regression analysis indicated that CYP2D6 (188C > T) genotype was an independent predicting factor for HVPG response to propranolol ($P = 0.033$).

Conclusions: CYP2D6 (188C > T) gene polymorphisms influence the hemodynamic response to propranolol in this population of Chinese Han patients with gastroesophageal varices. However, HVPG response cannot be completely predicted from CYP2D6 and β 2-AR gene polymorphisms.

O-248

Characterization of gene expression profile in HBV-related liver fibrosis patients

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Background: HBV-related liver fibrosis (LF) causes high morbidity and mortality worldwide. However little is known about the gene expression profile in the pathophysiologic change of LF and their relationship with the degree of decompensation.

Methods: Liver specimens were obtained from 109 treatment naive hepatitis B patients. Total RNA of these specimens were extracted to perform microarray experiments using Affymetrix HG U133 Plus 2.0 arrays. Differential expressed genes (DEGs) were screened based on fibrosis severity according to Scheuer Scoring. Functional analysis including pathway, GO, PPI network and gene-miRNA network were performed. In addition, we applied various statistical methods including trend test, GSEA, PCA and WGCNA to analyze the hub genes through the progress of fibrosis.

Results: 312 LF related DEGs were screened out, including 296 up- and 16 down-regulated genes. Among these genes, the expression of *CXCL6*, *ITGBL1* were significantly changed and demonstrated to be positively correlated with fibrotic progression by trend test. GSEA and Pathway analysis showed that cytokine-cytokine receptor pathway and Toll like receptor pathway were activated. MicroRNA has-miR-34a-5p and its interactive DEGs formed a significant cluster in the network analysis. WGCNA showed that *CXCL6* and *ITGBL1* were highly ranked hub genes.

Conclusions: This was the first large-scale study of gene expression profile in HBV-related LF patients. Preliminary results showed that multiple pathways and genes were involved in the fibrotic mechanism, in which has-miR-34a-5p, *CXCL6* and *ITGBL1* were crucial. Further experiments are needed to support our conclusions.

O-249

Risk and adverse outcomes of stroke in patients with liver cirrhosis: two nationwide studies**Chien-Chang Liao¹, Chun-Chuan Shih²**¹School of Medicine, Taipei Medical University, Taiwan, Taipei;²School of Chinese Medicine for Post-Baccalaureate, I-Shou University, Kaohsiung, Taiwan

Background: The association between liver cirrhosis (LC) and risk of stroke was not completely understood. This study evaluated stroke risk and post-stroke outcomes in patients with LC.

Methods: We identified 3955 adults aged 20 years and older newly diagnosed with LC using the Taiwan National Health Insurance Research Database from 2000 to 2005. Comparison cohort consisted of 15,820 adults without LC randomly selected by frequency matching in age and sex. Events of new-onset stroke were identified from medical claims during the follow-up period in 2000–2009. Adjusted hazard ratios (HR) and 95 % confidence interval (CI) of stroke associated with LC were calculated in the multiple Cox proportional hazard model. Another nested stroke cohort study of 21,267 hospitalized stroke patients analyzed for adjusted odds ratios (ORs) and 95 % CIs of adverse events after stroke among patients with and without LC between 2000 and 2010.

Results: The incidences of stroke for people with and without LC were 6.1 and 4.3 per 1000 person-years, respectively. Compared with non-cirrhotic cohort, the adjusted HR of stroke was 1.55 (95 % CI 1.28–1.87) for LC patients. Previous LC was associated with risks of epilepsy (OR 1.30, 95 % CI 1.09–1.56), admission to intensive care unit (OR 1.23, 95 % CI (1.14–1.32), and mortality (OR 1.83, 95 % CI 1.63–2.05) after stroke.

Conclusion: LC was associated with higher risk of stroke and patients with LC had more complications and mortality after stroke. Stroke prevention and attention to post-stroke adverse events are needed for this susceptible population.

O-250

Marrow cellularity & liver disease severity predicts regenerative response to therapy in cirrhotics**Lovkesh Anand, Chandan Kedarisetty, Chhagan Bihari, Awinash Kumar, Amrishi Sahney, Smriti Shubham, Dhananjay Mathur, Sheetal Rooge, Rakhi Maiwall, Anupam Kumar, Shiv Kumar Sarin**

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Introduction: Mobilization of stem cells utilizing combination of exogenous growth factors has a promising role in the management of decompensated liver diseases, but not all patients show an adequate response. We compared various clinical and Bone Marrow (BM) parameters to predict responsiveness.

Methods: This randomized controlled double blind trial was conducted on 60 consecutive alcoholic or cryptogenic decompensated cirrhosis (DC) patients aged 18–65 years who underwent BM examination and transjugular liver biopsy (TJLB) at baseline; received either G-CSF (5 µg/kg s/c, 22 doses) and erythropoietin (500 IU/kg, 16 doses) combined or G-CSF and placebo for 2 months. The regenerative response was assessed both clinically and histologically.

Results: 41 patients had paired liver biopsies. 70.7 % (n = 29) of the patients had clinical (reduction in CTP score > 1) and/or histological response (Table 1) and were defined as responders. No relation of

age, aetiology of cirrhosis, treatment administered (combination or single growth factor) or presence of co-morbidities seen with the regenerative response. On univariate analysis, severity of decompensation [baseline CTP Score ≤9, MELD score ≤15.5 and MELD Na score ≤18.5] and baseline BM markers [lower osteocytes, decreased perivascular and BM fibrosis, decreased neo-vascularisation, greater CD34 positive cells and higher Hematopoietic stem cells (HSC)] were significantly associated with responsiveness. On multivariate analysis, baseline MELD and % of HSC were found to be independent predictor of regenerative response with AUC of 82.25 (Table 1; Fig 2).

Conclusion: Early DC show a better response to growth factors. Performing a baseline BM analysis prior to initiation of regenerative therapy portrays a window to the regenerative capacity of the liver and thus can guide selecting appropriate candidates.

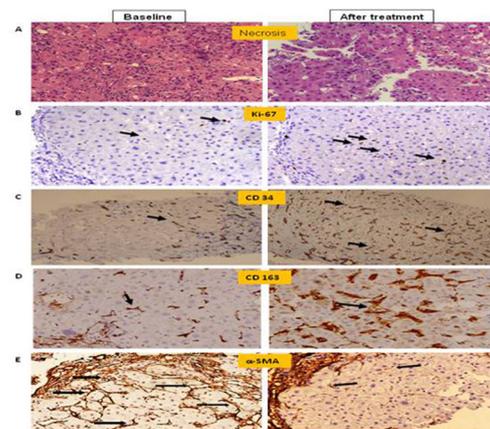


Figure 1: Paired liver biopsies showing A) decrease in parenchymal necrosis post regenerative therapy; B) Increased Ki-67 staining in lobular parenchyma post treatment; C) Increased CD34 staining in lobular parenchyma post treatment; D) Increased M2 Macrophage CD 163 staining in lobular parenchyma post treatment 200X; E) Decrease in α-SMA staining (brown staining) post treatment; 200X.

	Parameter	Odds ratio	95% C.I.	P Value
Clinical parameters	MELD (> 15.5)	5.70	1.254 - 25.918	0.024
	CD34	1.253	0.979 - 1.604	0.07
Baseline BM parameters	HSC (>0.4)	84.33	7.848 - 906.20*	<0.001

* Wide CI due to very few events in the negative group

Table 1: Multivariate analysis of clinical parameters and baseline BM parameters to predict regenerative response

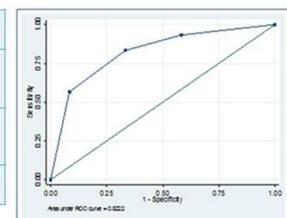


Figure 2: Receiver Operating Characteristic (ROC) curve of the model combining HSC and MELD score as predictors for liver regeneration

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Identification of a SNP in BMP6 implicated in iron accumulation in 3 Asian patients**V. Nathan Subramaniam¹, Cameron McDonald¹, Gautam Rishi¹, Lesa Ostini¹, Darrell Crawford², Paul Clark², Hanlon Sia³, Daniel Wallace¹, V Nathan Subramaniam¹**¹QIMR Berghofer Medical Research Institute, Brisbane, Australia;²Faculty of Medicine and Biomedical Sciences, The University of Queensland, Brisbane, Australia; ³ICON Cancer Care, Gold Coast, QLD, Australia

Background: Hereditary hemochromatosis (HH) is relatively common in individuals of northern European descent, with disease

prevalence greater than 1:200 in countries with high levels of northern European ancestry. The majority of HH cases in these countries are caused by mutations in the HFE gene. In non-European populations however, in particular those of Asian origin, mutations in the HFE gene are rare, and HH is more commonly caused by mutations in other genes involved in the regulation of iron homeostasis, termed non-HFE HH.

Methods: We have developed a novel targeted next-generation sequencing platform to examine the molecular basis of atypical iron overload disorders. We performed targeted next-generation sequencing of 39 genes associated with iron regulation in 3 subjects of Asian origin presenting with increased serum ferritin and evidence of hepatic iron accumulation. Molecular characterization was performed by immunofluorescence microscopy of transfected cells and Western blotting.

Results: We report the identification of a duplication of the glutamine amino acid at position 118 (p.Q118dup) in Bone Morphogenetic Protein 6 (BMP6), a protein shown to have a central role in the regulation of iron metabolism. Preliminary functional characterization indicates that the polymorphism affects processing and secretion of the protein.

Conclusions: This is the first report implicating polymorphisms in the BMP6 gene with clinical iron overload in Asian patients.

O-252

Iron chelator suppresses hepatocarcinogenesis through mitophagy induction in STAM® mice

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Background and aim: Mitochondrial quality control (mitophagy) has been shown to be suppressed in nonalcoholic steatohepatitis (NASH), which is related to disease progression. Therefore, the restoration of mitophagy is a critical therapeutic intervention for preventing disease progression in NASH. On the other hand, iron loss has been reported to trigger PINK1/Parkin-independent mitophagy. The aim of this study was to determine whether iron chelation restores mitophagy suppressed in NASH.

Methods: Huh7 cells were treated with deferiprone (DFP), iron chelator. STAM® mice were administered DFP for 3 months.

Results: DFP treatment increased the expression of LC3-II and decreased the expression of p62 in dose-dependent manner, but did not affect translocation of Parkin to mitochondria in Huh7 cells. Electron microscopy also revealed that DFP significantly increased the number of mitophagosomes. DFP decreased reactive oxygen species (ROS) production, but not ATP production, and suppressed hepatic steatosis, hepatic fibrosis and liver tumor development in Huh7 and/or STAM mice liver. The inhibitory effects of DFP on ROS production and disease progression in STAM® mice were cancelled by knockdown of Atg5, autophagy-related gene. We next measured mitochondrial ferrous iron content in Huh7 cells, using mitochondrial iron-specific fluorescent probe. DFP decreased mitochondrial ferrous iron content in a dose dependent manner.

Conclusion: These results indicated that iron chelation suppresses hepatocarcinogenesis through mitophagy induction. The mechanisms underlying mitophagy induced by iron chelation remains to be elusive, but decrease in mitochondrial ferrous iron may be related to mitophagy induction since mitochondria play a critical role in cellular iron metabolism.

O-253

Elevated 53-binding protein 1 nuclear foci expression in the NASH liver

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Background: DNA damage response could result in genomic instability, leading to transformation to cancer. However, the presence of genomic instability in the NAFLD liver is largely uninvestigated. Our aim was to assess the double-strand breaks by nuclear expression of DNA damage response protein 53-BP1 in hepatocytes of NAFLD liver and free fatty acid-treated primary hepatocytes.

Methods: Isolated rat primary hepatocytes were exposed to saturated free fatty acid palmitate. Twenty three human liver biopsy paraffin embedded samples, including 5 from normal livers, 5 from simple steatotic livers, and 13 from livers with NASH were studied by co-immunofluorescence with the anti-53-BP1 and hepatocyte marker.

Results: Palmitate treatment in the rat primary hepatocytes induced a significant increase in the number of 53-BP1 nuclear foci. In the human liver biopsy tissue, the number of 53-BP1 nuclear foci in the hepatocytes was increased by approximately 6 folds in the NASH livers compared to the simple steatotic livers. Number of foci more than 1µm in diameter was also increased in NASH patients' liver. These 53-BP1 nuclear foci were evenly distributed throughout hepatic zone 1, zone 2, and zone 3. The rate of hepatocytes with large foci was positively associated with age and serum hyaluronic acid in NAFLD patients, and was negatively associated with PLT.

Conclusion: Genomic instability may lead to accumulation of genomic alterations, which possibly contribute to progression to cirrhosis and/or malignancy in NAFLD patients. Immunofluorescence examination of 53-BP1 expression might be beneficial for estimating the degree of genomic instability in NASH.

O-254

Effect of metformin on disease progression of NASH-cirrhosis with diabetes/insulin resistance

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Background and aims: Metformin reduces insulin resistance, central in progression of NASH-cirrhosis. In a retrospective study, continuation of metformin after diagnosis of cirrhosis was safe and reduced the risk of death by 57 %. No prospective study has evaluated safety/efficacy of metformin in preventing progression of NASH-cirrhosis. We studied the same using clinical and hemodynamic parameters.

Patients and methods: Of the 332 patients with NASH-cirrhosis seen in outpatient clinic, 98 with Child's A/B cirrhosis were randomized (NCT02234440) to metformin (n = 50) or conventional (n = 48) arm with same dose of exercise and diet. The primary end point (PEP)

(at least 10 % reduction in HVPG) was assessed after 12 months of follow-up. Safety profile of drug, reduction in LSM/CAP, decompensation/complication were recorded three monthly.

Results: Patients in both arms were comparable at baseline. Till now, PEP could be assessed in 45 patients (21 in metformin, 23 in conventional arm). Fourteen (66.6 %) in the former and one (4.4 %) in conventional arm could achieve PEP. In 12 months, average reductions in HVPG (%), weight (%), LSM (kPa) and CAP (dB/m) in metformin vs conventional arm were 19.4, 10.4, 9 and 53 vs -13.4, 2.7, 2.3 and 20.9 respectively. Metformin was safe and was not associated with hypoglycaemia/lactic acidosis.

Conclusions: Metformin along with lifestyle modifications could be an effective treatment in the management of carefully selected early NASH-cirrhosis. Reduction in HVPG/weight/LSM by metformin in this study would translate into reduction in progression of CLD/HCC development. The long-term results of the entire cohort would help in better understanding and stronger recommendations in near future.

O-255

An essential role of regulatory T cells: prevention of alcohol-induced hepatic steatosis in mice

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Background: Alcoholic fatty liver <AFL> renders the liver more susceptible to the development of advanced alcoholic liver diseases <ALD>. However, whether Tregs play a role in the pathogenesis of AFL has not been studied.

Methods: The Leiber-DeCarli diet containing alcohol were fed to C57BL/6 mice for 6 weeks and the alcohol-feeding mice were injected with CD4 + CD25 + T cells or control CD4 + CD25– T cells intravenously from the fourth week, twice at 1-week intervals. Functional effects of Tregs on lipid metabolism, oxidative stress, and macrophage activation were examined.

Results: Chronic alcoholic feeding induced liver steatosis and increased serum alanine aminotransferase accompanied with the depletion of hepatic Tregs, but these liver damages were blunted by Treg adoptive transfer. The enhanced expression of nuclear Sterol regulatory element-binding protein 1c <SREBP1c>, and its downstream genes, were induced in alcohol-fed mice but not in Treg-transferred mice, in which hepatic oxidative stresses were also ameliorated. Moreover, Tregs increased the level of phosphorylated adenosine monophosphate-activated protein kinase <AMPK>, peroxisome proliferator-activated receptor <PPAR> α in nucleus, and its downstream genes for fatty acid metabolism, which were inhibited by alcohol-feeding. Furthermore, Tregs inhibited MCP-1 and TNF- α ; overproduction and macrophage activation in livers. In vitro, Treg suppress the expression of MCP-1, TNF α and CD14 on monocytes/macrophages undergone LPS and alcohol co-treatment. These suppression was markedly abrogated by neutralizing anti-IL-10 mAbs. **Conclusions:** Our results indicate that Tregs suppress the development of <AFL>, in part

through modulating lipid metabolism, oxidative stress and macrophage pro-inflammatory response.

O-256

HCBP6 mediates the reciprocation between intracellular total cholesterol and cell autophagy

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Objectives: Explore whether mir-185 and mir-122 mediated HCBP6 expression maintaining of cholesterol (TC) and triglyceride (TG) homeostasis and whether there is reciprocation between TC and autophagy.

Methods: (1) Detect the level of HCV core protein binding protein 6 (HCBP6) of fatty mice hepatic tissue. (2) Measure the TC and TG levels of HCBP6-overexpressed or HCBP6-silenced in L02 and HepG2 cells. (3) Measure HCBP6 expression in cells were treated with and without the cholesterol-depleting or cholesterol-overloading agent, HPCD or cholesterol, respectively. (4) Measure the autophagy of HCBP6-overexpressed or HCBP6-silenced cells. (5) In cells with and without the cholesterol-depleting or cholesterol-overloading agent, HPCD or cholesterol, respectively, we compared the level of autophagy and the transcriptional and translational level of HCBP6. (6) Investigate TC and HCBP6 level by using 3-MA or CQ to block the autophagy pathway in HCBP6-overexpressed or HCBP6-silenced HepG2 cells.

Results: (1) Decreased HCBP6 expression level in the liver of high-fat diet mice. (2) Mir-185 and mir-122 mediated HCBP6 expression maintaining of cholesterol homeostasis by down-regulating SREBP2/HMGCR and SREBP1c/FASN. (4) HCBP6 induced autophagy. (5) When reduce or increase TC level, a significant reduction or increase of HCBP6 was observed by time and dose dependent manners, respectively. (6) After silenced HCBP6 in cholesterol-overloading cells, autophagy was decreased. (7) When autophagy pathway was blocked by 3-MA or CQ, HCBP6 was down-regulated, and TC level was increased.

Conclusion: (1) HCBP6 is the sensor and oscillator in the cholesterol homeostasis. (2) HCBP6, also high level of TC, induces autophagy through Beclin-1-independent pathway. (3) HCBP6 mediates the reciprocation between intracellular TC and autophagy.

O-257

The effect of female hormones on NAFLD in p62 knockout mice

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Background: The progression of nonalcoholic fatty liver disease (NAFLD) is accelerated in postmenopausal women. This finding implicates an important role for the female hormone in NAFLD, but the details of that mechanism are still unclear. Therefore we generated

p62 knockout mice (*p62*-KO). *p62*-KO are the model of metabolic syndrome because of obesity, hypertension and insulin resistance.

Objective: To confirm why there are gender differences in NAFLD progression, we focused on the effect of female hormones on NAFLD progression in *p62*-KO.

Methods: we compared male and female *p62*-KO regarding body weight, food intake and liver pathological findings. We then performed bilateral ovariectomy (OVX) or sham operation (SO) on 9-week-old female *p62*-KO and investigated body weight, food intake and liver pathological findings. Moreover, we investigated body weight and food intake of male *p62*-KO implanted with estradiol (E2) release pellets or placebo vehicle.

Results: Body weight and food intake of male *p62*-KO increased gradually from young; however, females got fat rapidly from around 30 weeks. Moreover, steatosis and inflammation levels of the liver in males were more progressed than females at the same age. Body weight and food intake of OVX were more than that of SO. Moreover, steatosis levels in OVX were more progressed than that of SO. Body weight and food intake levels of male *p62*-KO injected with E2 pellets were less than those injected with placebo.

Conclusions: It is suggested that female hormones suppress NAFLD progression on *p62*-KO. Female *p62*-KO are useful for the model animals of NAFLD on postmenopausal women.

O-258

***p62*:Nrf2 double knockout mice develop steatohepatitis through the serum endotoxin overload**

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Background: Non-alcoholic steatohepatitis (NASH) is a progressive liver disease leading to liver cirrhosis and cancer. Thus, we generated double knockout (DKO) mice without *p62*, a gene to regulate signal transduction and food intake or *Nrf2*, an important transcription factor for anti-oxidative stress.

Aim: To confirm a hypothesis for bacteria-induced metabolic liver disease, we focused on the endotoxin produced by Gram-negative bacteria. The aim of this study was to elucidate the regulatory role of *p62* and *Nrf2* in pathogenesis of NASH using the DKO mice.

Methods: The liver tissues were analyzed for pathology. To determine fecal microbiota composition in mice, we performed PCR of the bacterial 16S rRNA genes followed by T-RFLP. We also measured endotoxin in the serum and feces. Intestinal permeability was evaluated by measuring the permeability of FITC-Dextran.

Results: In DKO mice, severe NASH was developed with the pathological state of steatohepatitis in terms of fatty changes, inflammation, and fibrosis after 25 weeks age. Serum endotoxin levels were significantly higher in DKO mice than that in WT mice. Furthermore, intestinal permeability, assessed by measuring plasma levels of FITC-Dextran administered by an oral load, gram-negative bacteria levels and endotoxin in feces were all increased significantly in DKO mice.

Conclusions: The overload of serum endotoxin induced by the modification of intestine microbiota and acceleration of intestinal permeability was considered to be important for the progression of NASH in the DKO mice. These findings suggest *p62* and *Nrf2* as promising targets toward developing new options for prevention and treatment of NASH.

O-259

Adenosine 2A receptor deficiency exacerbates NAFLD in both HFD-fed and MCD-fed mice

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Adenosine 2A receptor (*A_{2A}R*) mediates the anti-inflammatory activity of adenosine, and has a protective role in non-alcoholic fatty liver disease (NAFLD). However, the mechanisms underlying *A_{2A}R* actions remain largely unknown. Here we investigated the effects of *A_{2A}R* deficiency on aspects of NAFLD in both obese mice and lean mice upon feeding a high-fat diet (HFD) and a methionine- and choline-deficient diet (MCD), respectively. In obese mice, hepatic steatosis and proinflammatory responses were evident as indicated by changes in liver histology and inflammatory signaling and cytokine expression in relative to those in low-fat diet-fed mice. Furthermore, the severity of HFD-induced aspects of NAFLD in *A_{2A}R*-deficient mice was much greater than in wild-type (WT) control mice, which was accompanied with increased body weight, adiposity, and adipose tissue infiltration of proinflammatory macrophages in HFD-fed *A_{2A}R*-deficient mice. Unlike HFD-fed mice, all MCD-fed mice displayed lean phenotype, which was attributable to a significant decrease in food intake. In the absence of adiposity, *A_{2A}R*-deficient mice still displayed a greater increase in the severity of hepatic steatosis and proinflammatory responses compared with WT mice. In an in vitro system, *A_{2A}R*-deficient macrophages showed an increase in proinflammatory responses compared with control cells. In addition, co-culture of WT primary mouse hepatocytes with *A_{2A}R*-deficient macrophages caused significant increases in hepatocyte fat deposition and in hepatocyte proinflammatory responses compared with WT hepatocyte-macrophage co-cultures. Taken together, these results suggest that *A_{2A}R*-deficiency-associated inflammation is sufficient enough to bring about NAFLD aspects. Thus, *A_{2A}R* protects against NAFLD mainly through its anti-inflammatory activity.

O-260

Curcumin effectively inhibits CXCL10 associated pathogenesis in the liver of NASH-HCC mice model

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Background and aims: CXC motif chemokine ligand 10 (CXCL10), a proinflammatory cytokine has been implicated in non-alcoholic steatohepatitis (NASH) pathogenesis based on animal and human data. The study aim was to investigate the effect of curcumin on the expression of CXCL10 in the NASH liver of a novel mouse model.

Methods: Neonatal C57BL/6 male mice exposed to low-dose streptozotocin (STZ) and were fed high-fat diet (HFD) at the age of 4 weeks and Continued for 10 weeks, curcumin was given at 100 mg/kg by oral gavage daily after 6 weeks of HFD feeding and continued for 4 weeks along with HFD feeding.

Results: In this investigation, curcumin improved the NASH pathology and histological changes with steatosis ($p < 0.001$) and fibrosis ($p < 0.05$) in the liver of NASH mice. NASH mice developed steatohepatitis with a higher hepatic CXCL10 expression ($p < 0.01$) which was significantly reduced by curcumin treatment ($p < 0.001$). Curcumin also reduced important pro-inflammatory and inflammatory cytokines (IL-1 β , IFN γ) and activation of the NF- κ B pathway in the liver of NASH mice. Curcumin treatment also improved the hepatic upregulation of the lipogenesis (SREBP1c) and oxidative stress (CYP2E1 and C/EBP β) in NASH mice. Apoptotic and anti-apoptotic signaling proteins were significantly increased and decreased respectively in NASH mice, but curcumin treatment markedly altered these expressions.

Conclusions: Finally, our results indicate that curcumin has the potential to protect the liver by modulating CXCL10 mediated inflammation, lipogenesis, oxidative stress and apoptosis and provides a novel therapeutic strategy for the NASH liver.

O-261

L-carnitine prevents liver fibrosis and preneoplastic lesions in rat model induced by CDAA and DEN

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Background: NASH can progress to advanced liver fibrosis and ultimately make HCC. L-carnitine was synthesized in the body from the amino acids and proposed as the antioxidant for kidney disease and many body conditions. The aim of this study is to investigate whether L carnitine has any effects on the enlargement of liver fibrosis and preneoplastic lesions.

Methods: The effects of L-carnitine were examined using the choline deficient L-amino acid-defined <CDAA> diet-induced rat model. N-Diethylnitrosamine <DEN> 10 mg/kg was injected intraperitoneally once a week. One group received CDAA diet with L carnitine <200 mg/kg/day> and DEN injection, the other group was CDAA diet and DEN injection without L-carnitine as controls. After 16, 20 weeks, Liver fibrosis was analyzed by Azan, Sirius-red, α SMA, ED-1 <CD68> expression. The preneoplastic lesions was assessed by glutathione S-transferase placental form <GST P> expression. The change of laboratory data was analyzed. Type I procollagen, TIMP1,2, α SMA, TGF β R1, MMP2,9,13, AFP, TNF α , MCP1, IL6R, EpCAM mRNA expression were analyzed using RT-PCR and DNA array.

Results: After 16 weeks, L-carnitine prevented liver fibrosis by Azan, Sirius-red expression < $p < 0.05$ >. L-carnitine reduced the area of GST-P positive lesions as preneoplastic lesions < $p < 0.05$ >.

Administration of L-carnitine significantly reduced levels of serum AST < mean value: L-carnitine 239.3 vs Control 391.3 IU/l, $p < 0.05$ > , serum ALT < mean value: L carnitine 242.8 vs Control 326.3 IU/l, $p < 0.05$ > , serum albumin < mean value: L-carnitine 4.5 vs Control 3.9 g/dl, $p < 0.05$ >. L-carnitine significantly inhibited Type 1 procollagen, TNF α , IL6R, AFP, EpCAM mRNA expression <all of $p < 0.05$ >.

Conclusion: Our results indicated that L-carnitine prevented liver fibrosis and inflammation, and protected liver function. We suggested that L-carnitine will be the new drug for NASH as pre-emptive medical care.

O-262

A novel, highly potent, non-bile acid FXR agonist for the treatment of NASH and cholestasis

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The Farnesoid X Receptor (FXR) is a bile acid (BA)-activated nuclear receptor and a key regulator of bile acid metabolism. FXR agonists decrease hepatic triglyceride synthesis leading to reduced steatosis; inhibit hepatic stellate cell activation reducing liver fibrosis; improve gut barrier function which reduces hepatic inflammation and increase FGF19 leading to improved hepatic insulin sensitivity. In clinical studies, obeticholic acid (OCA), a bile acid-derived FXR agonist, showed efficacy in both Primary Biliary Cirrhosis (PBC) and non-alcoholic steatohepatitis (NASH) patients; however OCA treatment was associated with increased pruritus. Here we describe a novel, non-bile acid FXR agonist LJN452. In both biochemical and cellular assays, LJN452 showed high potency (EC50 = 0.3 nM) on FXR with no activity on other nuclear receptors or TGR5. LJN452 stimulated expression of FXR target genes in primary human hepatocytes, and in rat liver and intestine in vivo, and increased circulating FGF15 protein levels in a dose- and time-dependent manner. In a rodent model of cholestasis, LJN452 effectively reduced liver damage and fibrosis. Due to the non-bile acid structure of LJN452, lack of TGR5 activity and very low peripheral exposure a reduced side effect profile relative to OCA may be observed. These preclinical data describe a novel, non-bile acid derived FXR agonist LJN452 which has the potential for a best-in-class profile in PBC and NASH.

O-263

Laennec® can improve type 2 diabetes complicating with NASH through normalizing iron metabolism

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Background: Recently it was elucidated that hepcidin, the principal regulator of iron metabolism, could express in pancreatic β cells, of which level could be regulated by iron. This fact means that the pancreas may contribute to the iron metabolism. In the cases of type 2 diabetes patients complicating with NASH, remarkable declines of serum ferritin and HbA1c were observed after treating with Laennec® (derived from human placenta). Then, we examined whether

Laennec® could restore the pathological background of type2 diabetes through regulating iron metabolism in NASH cases.

Methods: We divided 62 NASH cases (all with DM, liver biopsied) into two groups retrospectively. Laennec®-treated 31 cases were treated with the infusion of 2 ampules (224 mg) of Laennec®1–2 times/W, in addition to the ordinary liver supporting. Serum ferritin, ALT and HbA1c were measured, and liver re-biopsy was carried out to evaluate changes of iron deposition in 17 cases of NASH patients. **Results:** By infusing Laennec®serum ferritin level declined from 274.2 ± 310.4 ng/ml (before medication) to 58.3 ± 42.7 (after) (Wilcoxon $P < 0.01$) in NASH patients. Serum ALT also declined from 54.3 ± 20.1 IU/L to 24.8 ± 15.4 ($P < 0.001$). HbA1c level improved from 6.3 ± 1.1 % to 5.6 ± 0.7 ($P < 0.01$). When compared these results in two groups, the changes observed in Laennec®-treated group were significantly larger than non-treated group (Mann-Whitney $P < 0.05$). In multiplex-logistic analysis, the improvement of iron deposition in the liver correlate significantly with the decline of serum ferritin ($P < 0.01$).

Conclusions: The improvement of type 2 diabetes complicating with NASH by the administration with Laennec® suggests the importance of iron regulation on refractory type 2 diabetes which shows the presence of hyperferritinemia.

O-264

Share wave velocity fits the risk assessment of HCC development especially in NAFLD

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Aim: To evaluate the performance of share wave velocity (SWV) for the risk assessment of hepatocarcinogenesis in various chronic liver diseases.

Method: Virtual touch quantification was measured three times in each of the four liver segments in 58, 126, 38, and 180 of HBV, HCV, alcoholic liver disease (ALD), and, nonalcoholic fatty liver disease (NAFLD) patients including 23, 45, 15, and 9 cases with hepatocellular carcinoma (HCC), respectively, and the median value was statistically analyzed.

Results: The SWVs were 1.20 (interquartile range: 0.90–3.11), 1.45 (0.86–4.05), 2.05 (1.14–4.03), and 1.22 (0.79–3.73) m/s in non-HCC patients and were 1.70 (1.04–3.17), 2.47 (1.14–4.16), 2.58 (1.24–4.31), and 3.27 (2.20–3.74) m/s in HCC patients suffering from HBV, HCV, ALD, and, NAFLD, respectively. The SWV was significantly faster in the patients with HCC than without HCC except for ALD, in which the SWV distributed evenly between cases with or without HCC. The area under the receiver-operating characteristic curve to differentiate HCC from non-HCC cases was the largest in NAFLD of 0.963 followed by 0.795 in HCV leading to sensitivity and specificity of 88.9 and 87.7 % in NAFLD at 2.21 m/sec. Seven NAFLD cases without HCC showing SWV over the cut-off value could be reevaluated by MRI with an interval of one year or longer developed HCC or non-hypervascular nodule in 2 or 1 cases.

Conclusion: These results strongly suggest that SWV measurement is useful for the risk assessment of HCC development especially in NAFLD cases.

O-265

An essential role of nuclear receptors in hepatic lipid metabolism in hypertensive rat with NASH

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Background and aim: Hypertension is reported to be one of critical factors in the development of hepatic steatosis and steatohepatitis. Nuclear receptors, such as LXR and FXR, also play an essential role in hepatic lipid and bile acid metabolism. The aim of this study is to investigate whether hypertension modulates the function of nuclear receptors using a hypertensive rat.

Methods: 6–8 week-old spontaneously hypertensive rat (SHR) and Wistar-Kyoto (WKY) rats as normotensive controls were fed either normal chow or CD diet for 5 weeks ($n = 6$ /group), and gene expression of nuclear receptors (LXR and FXR) and their target genes were evaluated.

Results: CD diet induced marked hepatic steatosis in SHR as compared to WKY rats. Consistent with reduced expression of LXR (–75 %), the gene expressions of CYP7a1 (–65 %), CYP8a1 (–68 %), and CYP27a1 (–53 %), the important regulator for bile acid synthesis, were significantly reduced in SHR on CD diet. In addition, the expression of mRNA for canalicular membrane transporter ABCG5/G8 (–70 %), which are required for cholesterol secretion was down-regulated. Together with reduced expression of FXR (–79 %), its target genes of ABCB11 (–80 %) and ABCB4 (–81 %), which are requisite for bile acid secretion or phosphatidylcholine secretion were markedly reduced in SHR on CD diet. **Conclusion:** Hypertension was a strong exacerbation factor in liver steatosis. LXR and FXR were down-regulated in hypertension-related NAFLD animal model. These data suggest nuclear receptor ligands are promising agents for the future strategy of NAFLD associated with hypertension.

O-266

Screening for fatty liver: characteristics of patients with hepatic steatosis without elevated ALT

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Background: Early identification of patients with fatty liver may help improve the prognosis. Measurement of serum ALT levels is standard method to detect fatty liver disease; however, there are a problem associated with this marker in terms of its sensitivity. Development of further methods to detect fatty liver is now prioritized. Therefore, our **Aim** in this study was to analyze clinical characteristic of patients with hepatic steatosis without elevated ALT levels.

Methods: From July 2014 through November 2014, 1325 patients were evaluated by ultrasonography. Non-elevation group was defined as patients with serum ALT levels < 30 IU/L. The presence of fatty liver was identified in 27 % of HBV carrier, 21 % in chronic hepatitis C, 24 % of auto-immune hepatitis, 24 % of primary biliary cirrhosis and 59 % of others. Then, a total of 504 patients of others were analyzed for the relationship between the prevalence of fatty liver and blood biochemical findings.

Results: Non-elevation of serum ALT levels was found in 48 % of fatty liver patients. In the comparison of clinical characteristic in the non-elevation group between patients with/without fatty liver, HbA1c was higher in patients with fatty liver (6.0 ± 0.1 %) than without fatty liver (5.6 ± 0.1 %, $p = 0.006$). Using a cut-off of 5.6 %, HbA1c showed a sensitivity of 81 % and a specificity of 65 %, respectively.

Conclusions: There are many cases of fatty liver without elevated liver enzymes in serum levels. Individuals with elevated HbA1c levels over 5.6 % should be examined for the presence of fatty liver disease by means of diagnostic imaging.

O-267

Factors associated with the increase in FIB-4 score: a longitudinal study

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Background and aims: Non-alcoholic fatty liver is associated with metabolic syndrome and cardio vascular diseases. It is also important as a liver disease because it can develop non-alcoholic steatohepatitis and progress to liver fibrosis. Using FIB-4 index, which has been established as a non-invasive marker for liver fibrosis, we aimed to determine factors associated with the development of fibrosis in the liver.

Methods: We included 6246 participants (3754 men and 2501 women; median age, 45 years old) whose FIB-4 score was less than 1.450 at baseline and who received follow-up study at least once between 2006 and 2011. Using Cox proportional hazard model, we evaluated the influence of metabolic syndrome-related factors on the increase in FIB-4 score to 1.659 or more.

Results: The total follow-up period was 12,987.1 person-years, during which 271 cases increased FIB-4 score. In individual analysis, male sex (hazard ratio, 1.585 [95 % confidence interval, 1.226–2.048]), glucose intolerance (1.495 [1.177–1.899]), dyslipidemia (1.393 [1.072–1.810]), hypertension (1.893 [1.491–2.403]), and alcohol consumption (1.448 [1.127–1.861]) were significant risk factors. In multivariable analysis, only hypertension remained statistically significant (1.686 [1.311–2.168]). Meanwhile, in 1941 participants with fatty liver at baseline, 74 cases increased FIB-4 score. Exercise was also a significant risk factor (1.921 [1.194–3.089]) besides hypertension (1.768 [1.065–2.933]). **Conclusions:** Hypertension was a significant risk factor for the development of liver fibrosis. Among cases with fatty liver, exercise at baseline was also associated with the development of liver fibrosis.

O-268

A common variant in the PNPLA3 in university students with non-alcoholic fatty liver disease

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The patatin-like phospholipase domain-containing 3 (PNPLA3) rs738409 (Ile148Met, C > G) gene polymorphism is one of the most important genetic determinants of non-alcoholic fatty liver disease (NAFLD). The impact of rs738409 on the prevalence of NAFLD in young adults has not been fully investigated. One hundred and twenty university male students with abnormality in the blood tests including serum liver enzyme levels in the entrance health checkup were enrolled. Genotyping of rs738409 was performed using the TaqMan SNP Genotyping Assay. The mean age \pm SD of the students was 19.5 ± 1.9 . Of 120 students, 78 (65 %) had elevated levels of alanine aminotransferase and 81 (68 %) had NAFLD. NAFLD was significantly more prevalent in obese students than in non-obese students (96 vs. 47 %, $P < 0.001$) and tended to be more prevalent in students with GG genotype than those with non-GG genotypes (79 vs. 63 %, $P = 0.078$). Multivariate logistic regression analysis revealed significant association between NAFLD and obesity (Odds ratio 1.39, 95 % confidence interval 1.21–1.60, $P < 0.001$) but not GG genotype (Odds ratio 1.09, 95 % confidence interval 0.92–2.78, $P = 0.097$). Although being underpowered due to limited number of cases, obesity but not rs738409 polymorphism is significantly associated with NAFLD in young adults, indicating the importance of life style rather than genetic background in the present setting. Further studies are needed to investigate the importance of PNPLA3 genotypes in the persistence and disease severity of NAFLD.

O-269

Hepatocyte transplantation using living donor reduced grafts

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Background: Hepatocyte transplantation (HT) has been indicated in patients with metabolic liver diseases and acute liver failure as a bridge or an alternative to liver transplantation. However, one of the problems of HT is the limited availability of donor tissue. In our center, hepatocytes were obtained from remnant tissue of left lateral segment graft from unrelated donors. The aim of this study was to report the experience of HT using cryopreserved hepatocytes from resected living donor liver tissue.

Methods: We experienced two cases of HT using hepatocytes from living donor. Patients were neonatal ornithine transcarbamoylase deficiency (OTCD) and neonatal carbamoyl phosphate synthetase I deficiency (CPS1D). The age at HT was at 11, 14 days of age in OTCD case, and 18, 20 and 22 days of age in CPS1D case, respectively. Double lumen catheter was inserted into left portal vein via umbilical vein. Tacrolimus and low dose of steroid were initiated as immunosuppression therapy after HT.

Results: HT was performed successfully in both cases. Total counts and mean viability of transplanted hepatocytes were 7.01×10^7 cells and 1.76×10^8 cells, 85.9 and 64.6 %, respectively. Although both cases experienced hyperammonemia episode after HT and required continuous hemodialysis and filtration, severe neurological

impairment did not presented. Both patients underwent living donor liver transplantation 5 months after HT. Postoperative courses were uneventful. No neurological sequelae related to hyperammonemia have been observed.

Conclusion: HT using the remnant liver of reduced grafts from living donor, a novel source of hepatocytes, was safe and useful.

O-270

A case of congenital hepatic fibrosis leading to hepatocellular carcinoma

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Background: Congenital hepatic fibrosis (CHF) is rare hereditary disease that is classified into the one of fibropolycystic disorders. Pathological features of CHF are fibrosis and irregularly dilated bile ducts in portal areas, but hepatocytes are almost preserved. Therefore, liver functions are apparently normal despite the presence of portal hypertension clinically.

Case report: It was the case of 38-year-old Japanese man with CHF with hepatocellular carcinoma (HCC). He was diagnosed as CHF in 2003 at the previous hospital and had received follow-up examination. In 2013, a liver tumor (16 mm) in left lobe was detected by ultrasonography and diagnosed as well differentiated HCC by tumor biopsy, then referred to our hospital. Additional examination with computed tomography and magnetic resonance imaging showed two HCCs in S2 (28 mm), S4 (24 mm). Both tumors were treated by laparoscopic radiofrequency ablation. Without any complications, he was discharged 3 days after the operation and now we have continued the follow-up observations.

Discussion: HCC is a rare complication of CHF, so only 3 cases are have been reported up to now. In all 3 cases, the onset age of HCC with CHF was relatively younger than usual type of HCC. Whether CHF could be precursor to HCC, remains unclear because of a few reports. But the previous one report, as well as our case, showed that multiple HCCs with CHF. From this feature, it could be some keys to explain the association of HCC and CHF.

O-271

Novel nucleoside analogs exert antiviral replication against HBV with drug resistance mutations

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Background and aim: Nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs) are effective against human immunodeficiency

virus type 1 (HIV-1) and hepatitis B virus (HBV) and both viruses often acquire resistance. The aim of this study is to achieve more-potent agents that offer profound viral suppression in NRTIs development.

Methods: Novel nucleoside analogues (NAs), 4'-C-cyano-2-amino-2'-deoxyadenosine (CAaA) and 4'-C-cyano-2'-deoxyguanosine (CdG), including 4'-ethynyl-2-fluoro-2'-deoxyadenosine (EFdA) congener exerts potent anti-HIV-1 activity, were generated for their anti-HIV-1/HBV activity. HBV clones with candidate Adefovir resistance (rtA181T/N236T) (ADVr), ETV resistance (rtL180M/M204V/S202G) (ETVr1) and (rtL180M/M204V/I163V/186T) (ETVr2; J Hepatol2015) mutations were determined for susceptibility to ETV, Adefovir, and novel NAs (CAaA and CdG) using southern blot. Moreover, we inoculated HBV wild strain or mutant (ETVr1) to chimeric mice with human hepatocytes, and administered CAaA and CdG for 14 days and monitored the serum HBV DNA titer by real-time PCR.

Results: Anti-HIV-1/HBV assays showed CAaA and CdG in lesser concentration decreased in both HIV-1/HBV DNA titers than ETV and EFdA. CAaA and CdG also strongly suppressed HBV replication with wild strain, ADVr, ETVr1 and ETVr2, suggesting that CAaA and CdG at low concentration were responsible for various mutants in contrast to commercialized NAs. Chimeric mice indicated CAaA brought about significantly greater levels of HBV reduction over days 3–14 ($p = 0.013$) in wild group, compared to ETV. CAaA also elicited significantly greater levels of HBV reduction by day 7 in ETVr1 group ($p = 0.0005$).

Conclusions: Two novel NAs, CAaA and CdG exert potent anti-HBV activity against both ETVr and ADVr, indicating new therapeutic option.

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Recurrent acute hepatitis induced by organic solvents

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A 35-year-old man with a 3-week history of worsening jaundice and flu-like symptoms was admitted to our hospital. Viral hepatitis serologies were all negative, as were findings of autoimmune factor. We considered liver transplantation, but the patient's liver function was recovered spontaneously. A liver biopsy showed massive infiltration of neutrophils but the cause of acute hepatitis was not identified. Four months after discharge, however, his liver function worsened again. We considered the possibility of antinuclear-antibody-negative autoimmune hepatitis and began treatment with steroid for which he responded effectively. Four months after discharge, the patient was admitted again for repeated liver injury. We started him on steroid pulse, which was not effective this time. We suspected organic-solvent-induced hepatitis considering his occupation: he started his own construction company just before the first admission where he was exposed more severely to organ solvents. Although urine test results for organic solvents were negative, a liver biopsy again revealed severe infiltration of neutrophils compatible with toxic hepatitis. After all, his liver function was improved spontaneously

again. Therefore, considering the pathology and detailed clinical course including that he became severely exposed to it since just before the first admission and the liver damage recovered spontaneously in the absence of the exposure, he was diagnosed with toxic hepatitis, and we had him strictly avoid organic solvents. He has been in good health without recurrence, suggesting the importance to rule out organic-solvent-induced hepatitis in acute hepatitis when the etiology is indeterminate.

O-273

The developing the detection method of Immune-mediated drug-induced liver injury

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Background: There are many challenges to make a clear diagnosis of drug-induced liver injury (DILI) since there is few clear evidence of pathology. The aim of this study is to analyze the immunological profiles in DILI patients and to investigate whether CYP3A4-mediated active metabolites could affect the immune response.

Methods: (1) We analyzed the proportions of immune cells in peripheral blood mononuclear cells (PBMCs) of DILI using BD FACS canto II, comparing 18 healthy controls with 6 acute viral hepatitis subjects. (2) PBMCs from DILI patients or healthy subjects were cultured with CYP3A4 expressing HepG2 cells and Asunaprevir using a transwell system. PBMCs from DILI subjects treated with asunaprevir were analyzed since asunaprevir-mediated DILI could be induced by immune-dysfunction. We collected PBMCs in the upper chamber and analyzed the proportions of the various kinds of immune cells at 48 h after co-incubation by BD FACS canto II.

Results: (1) There was significantly elevated proportion of MDSCs in the PBMCs of DILI and acute viral hepatitis, compared with those of healthy controls ($P < 0.0001$). The frequency of regulatory T cells (Tregs) in the PBMCs of DILI was higher than that of acute viral hepatitis and healthy controls ($P = 0.0862$). (2) The in vitro co-culture system could partially reproduce the immune profiles in some DILI patients.

Conclusion: These results demonstrated that immune-mediated DILI was associated with specific immune subsets. We might reproduce immune-mediated drug-induced liver injury with the in vitro co-culture system.

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Recombinant alfa-2b interferon plus ribavirin therapy in children aged 1 to 6 years with CHC

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Background/aim: Nowadays few data are available on the use of combination therapy with interferon and RBV in children. The aim

of the study was to evaluate the efficacy of alfa-interferon 2b in combination with RBV in children aged 1–6 years with unsafe injection-acquired CHC.

Methods: 154 children with CHC treatment aged 1–6 years (the median age was 2.92 years) and 65.6 % of them were male and 34.4 % were female and who were treated with subcutaneous alfa-interferon at a dose of 1–5 MIU/m² 3 times weekly in combination with oral RBV (15 mg/kg/day) for 48 weeks.

Results: 39.3 % of them were genotype 1b and 50.3 % genotypes 2a and 10.3 % undecided type. 66.7 % achieved RVR at 4 weeks and 99.4 % achieved EVR at 12 weeks. At the end of treatment and 96.8 % achieved SVR. Among genotype 1b 93.0 % achieved SVR and 98.6 % of genotype 2a achieved SVR. There were no significant statistics difference in SVR between male and female (98.0 vs 94.3 % and $p = 0.456$) and HCV genotype 2a and 1b (98.6 vs 93.0 % and $p = 0.160$). Leucopenia and neutropenia and hemoglobin concentration decrease and fever and platelet count decrease and rash were 96.1 % and 73.4 % and 90.9 % and 50.6 % and 1.9 and 4.5 % respectively. But only 12 (7.8 %) individuals developed thyroid autoantibodies. The IL-28B rs12979860 C/C and C/T accounted for 83.7 % (103/123) and 16.3 % (20/123) respectively. The rates of SVR were higher in patients with IL 28B C/C than C/T (99.0 vs 80 % and $p = 0.002$).

Conclusions: Combination of alfa-interferon with RBV is effective and safe in the treatment of CHC in children aged 1–6 years. IL-28B rs12979860 C/C is an independently predictive factor associated with higher SVR in children.

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Dysbiosis of the gut microbiota in primary sclerosing cholangitis (PSC) in children

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Background: Primary sclerosing cholangitis (PSC) is known to be complicated with inflammatory bowel disease in children, and dysbiosis of the microbiota is one theory of its pathogenesis. We investigated the gut microbiota of the pediatric PSC patients, and compared it with ulcerative colitis (UC) patients without PSC and healthy controls (HC) using 454 pyrosequencing of 16S ribosomal RNA genes and evaluated its bacterial composition.

Methods: 27 PSC patients (17 males, 10 females) whose median age at onset of symptoms was 6 years (2–15 years) and median present age was 12 years (3–24 years) were included in the study. All were complicated with some kind of colitis. Twelve patients [6 males 6 females, median age 12.5 years (10–22 years)] who have UC without PSC and 19 HC [8 males, 11 females, median age 7 years (3–23 years)] were included for comparison. After purifying the bacterial DNA of their feces, it was compared based on the molecular phylogeny of the 16S rDNA V1-V2 region.

Results: The numbers of operational taxonomic units of PSC patients and UC patients were less than those of HC, which suggests decreasing diversity of species in the gut microbiota. We used UniFrac to determine the microbial communities and PSC patients made a significantly different cluster with HC in both weighted and unweighted UniFrac, and also with UC patients in unweighted UniFrac.

Conclusion: These suggest that dysbiosis of the gut microbiota, which is different from that in UC patients, exist in PSC patients.

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Lack of infectivity of HBV in feces from patients with chronic hepatitis B virus infection

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Background: The infectivity of feces from patients with hepatitis B virus infection has not been established. The aim of this study was to determine whether feces from HBV carriers can be a source of HBV infection.

Methods: Thirty-three children and 17 adults who were chronically infected with HBV were enrolled. The levels of HBV DNA in the feces from these patients were quantified by real-time PCR, and the levels of fecal HBsAg were measured. Isolated human hepatocytes from chimeric mice with humanized livers were co-cultured with serum, tears and feces from the HBV carriers. Four chimeric mice were inoculated intravenously with sterilized feces from HBV carriers.

Results: HBV DNA was detected in the feces of 37 (74 %) of the 50 patients. The fecal HBV DNA levels ranged from 2.8 to 8.4 log copies/mL (mean \pm SD = 5.6 \pm 1.2 log copies/mL). A significant correlation was observed in the levels of HBV DNA between serum and feces (γ = 0.54, p < 0.05). Of the 13 HBV carriers, 7 (54 %) were positive for fecal HBsAg. The fecal HBsAg levels ranged from 0.06 to 1.0 IU/mL (median 0.28 IU/mL). HBV DNA was detected in the human hepatocytes co-cultured with serum and tears, but not in those co-cultured with feces. HBV DNA was not detected in the serum of the chimeric mice after oral or intravenous inoculation with sterilized fecal samples, which contained 5 log copies/mL of HBV DNA levels.

Conclusions: Feces from HBV carriers seem not to serve as an infectious vehicle for the transmission of HBV.

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The efficacy and safety of Telbivudine or Tenofovir in pregnancy for prevention of MTCT of HBV

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Background and aims: We evaluated the efficacy and safety profile in telbivudine (LdT) and tenofovir (TDF) in HBeAg-positive pregnant women with high viral load for preventing mother-to-child transmission (MTCT) in an open-label study.

Methods: 289 HBeAg-positive pregnant women with HBV DNA >10⁶ IU/mL enrolled were treated with NUCs from 20–38 weeks of gestation (264 in LdT, 25 in TDF). 171 cases who were unwilling to take antiviral drugs served as controls. All infants were vaccinated with recombinant HBV vaccine and hepatitis B immune globulin (HBIG). MTCT rate was determined by HBsAg and HBV DNA detection in 6 months after birth.

Results: Significantly lower HBV DNA levels were noted in the LdT/TDF group mothers when delivery. The rate of HBsAg positive and HBV DNA detectable in infants at 6 months was 0, 0 and 14.7 % in LdT, TDF and control group, respectively. The incidence of undetectable cord blood HBV DNA levels has significantly difference between NUCs treated groups and control group (LdT, 99.2 %; TDF group, 100 %, control group, 61.5 %, P < 0.05). No

obvious adverse events or complications were observed in all the mothers and infants.

Conclusions: Both LdT/TDF have showed excellent efficacy and safety profile in high viral load pregnant women and their infants, and meanwhile they were associated with significant reduction of MTCT.

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Long-term safety of infants born to LDT treated pregnancy with CHB during 2nd or 3rd trimester

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Aim: Evaluate the long-term efficacy and safety in infants born to telbivudine-treated mothers.

Methods: 210 pregnant women treated with telbivudine during the 20–32 week of gestation period were enrolled in this study. The efficacy and safety data of 214 infants aged 1 or above during March 2008–December 2013 were collected. The efficacy measure was the PT rate, which was established by levels of HBV_m and HBV DNA in the peripheral blood of infants at 7 month; and were continuously followed at 1, 2, 3, 4 and 5 year of age. The head circumference, height, weight, congenital abnormality rate and hospitalization rate of all infants at each age were also evaluated. 100 infants in telbivudine treatment group were randomly selected and were tested with Denver Developmental Screening Test.

Results: None of the 214 infants were infected with HBV. All infants in telbivudine had effective HBsAb. Based on the Chinese standard values of children's growth curve, there were no differences in mean values of the height, weight and head circumference in these 214 infants in all age groups born to telbivudine-treated mothers. Congenital abnormality rate was 0.934 % (2/214). SAE occurred in 20 infants (9.35 %) and required hospitalization. Of 100 infants selected for DDST, the qualified rate of DDST was 97.82 %, which was comparable to the rate of 92 % in normal Chinese children.

Conclusion: Infants born to telbivudine-treated mothers at the 2nd or 3rd trimester of pregnancy presented a normal growth and development during the follow-up up to 5 years of age.

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Comparison on the efficacy of endoscopic snare papillectomy with or without submucosal injection

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Background and aims: Injecting saline or diluted epinephrine to lift ampullary tumors during endoscopic snare papillectomy is not established, especially in view of achieving complete resection or

preventing post-procedural bleeding. We aimed to investigate the clinical efficacy of simple snaring method versus submucosal injection for papillectomy.

Methods: Patients with papillary lesions were randomized to undergo snare papillectomy (SP) or submucosal injection papillectomy (SMP). SMP group underwent papillectomy following submucosal injection of 1:10,000 epinephrine. Main outcome measures were complete resection rate and post-papillectomy complications.

Results: Forty patients with biopsy proven papillary adenoma were enrolled. Mean tumor size was 11.05 mm in SP group and 9.9 mm in SMP group. Mean procedure time was 20.3 and 18.6 min, respectively ($P = 0.411$). Average amount of injected diluted epinephrine solution in SMP group was 3.65 ml. Complete resection (negative lateral and deep resection margin on pathology) rate was 85 % (17/20) and 45 % (9/20), respectively ($P = 0.019$). Lateral and deep resection margin were more frequently positive in SMP group (0 vs. 5 patients). However, tumor recurrence at 3 months (after the procedure) was not different (10 vs. 10 %) despite initial difference in the prevalence of positive resection margin. Post-papillectomy bleeding developed in 35 % (7/20) and 45 % (9/20), respectively ($P = 0.519$). Delayed bleeding after 12 h was more prevalent in SMP group (5 vs. 15 %) albeit not statistically different. Post-procedure pancreatitis occurred in 20 % (4/20) and 30 % (6/20), respectively ($P = 0.465$). Severity of pancreatitis was also not different with no procedure related mortality.

Conclusions: Endoscopic papillectomy using simple snaring and submucosal injection were both effective methods. Although initial complete resection rate was higher in SP group, short term recurrence at 3 months was not different.

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Role of peroral cholangioscopy for diagnosis of intraductal papillary neoplasms of the bile duct

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Intraductal papillary neoplasms of the bile duct (IPNB) with or without mucin hypersecretion is considered an uncommon tumor and regarded as a biliary counterpart of intraductal papillary neoplasm of the pancreas. It is also well known that IPNB is often associated with superficial spread of tumors. Preoperative peroral cholangioscopic diagnosis is essential for treatment of IPNB, but, has not been elucidated properly yet.

Patients and methods: From December 1991 to November 2013 we underwent ERCP in 20 patients with IPNB (male/female 9/11, mean 67.0 years). Ten patients were IPNB with mucin hypersecretion (IPNB-M), 10 patients were IPNB without mucin hypersecretion (IPNB-NM).

Results: Peroral cholangioscopy underwent all patients except for one who died of decompensated biliary cirrhosis with massive hemobilia. Cholangiogram (ERCP) and peroral cholangioscopy diagnosed primary tumor location in 1 of 10, 7 of 9 patients with IPNB-M, in 8 of 10, 10 of 10 patients with IPNB-NM, respectively. Surgical resection underwent in 7 of 10 with IPNB-M, 10 of 10 with IPNB-NM, respectively. In 8 of 11 patients with superficial spread of tumor, peroral cholangioscopy made proper tumor-extent diagnosis preoperatively. Three patients with IPNB-M, who were diagnosed as inoperable due to major medical comorbidities, were died 3 to 6 months later due to biliary cirrhosis and/or cholangitis. Final pathological diagnosis were 7 adenoma, 11 carcinoma, 1 invasive carcinoma, 1 unknown, respectively.

Conclusions: Cholangiogram failed to delineate intraductal papillary tumor due to coexisting thick mucin or biliary sludge. Preoperative peroral cholangioscopy was useful for IPNB, especially that with mucin hypersecretion.

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Prognostic significance of modified Glasgow prognostic score in patients with gallbladder carcinoma

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Background: Gallbladder carcinoma (GBC) is one of the dismal digestive organ malignant cancers with a very poor outcome. The modified Glasgow Prognostic Score (mGPS), an inflammation-based prognostic score, has been found to be independently prognostic of survival in patients with cancer. The aim of this study was to investigate the clinicopathological prognostic variables including various inflammation-based prognostic scores for patients with GBC following curative intent surgical resection.

Methods: This was a clinical observational series of 54 patients with GBC who underwent surgical intervention between 1996 and 2014. Prognostic factors likely to influence overall and recurrence-free survival were assessed by univariable and multivariable analysis.

Results: R0 resection was achieved in 43 patients (79.6 %). The median age of the patients was 74 years (range 25–99 years). The majority of the patients (33, 61.1 %) were women. The overall and recurrence-free survival rates at 1, 3, and 5 years were 83.1, 63.3, 55.8, and 77.8, 58.4, 51.3 %, respectively. Incidental gallbladder carcinoma was detected in 18 patients (33.3 %). In multivariate analysis, postoperative complications ($P = 0.015$), curative resection ($P = 0.008$), histological type ($P = 0.003$), and mGPS ($P = 0.002$) were independent predictors of overall survival time. Postoperative complications ($P = 0.015$), curative resection ($P = 0.005$), histological type ($P = 0.002$), and mGPS ($P = 0.022$) were independent predictors of recurrence-free survival time.

Conclusions: Curative resection for GBC played an important role in long-term patient outcomes. The preoperative mGPS assessment could be used to select appropriate treatment strategies in patients with GBC.

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Progenitor repopulation by K7, K19 and Glutamine synthetase expression in hepatic vasculopathies

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Background: Hepatocyte progenitors are being recently shown to repopulate the areas of parenchymal extinction in regenerating liver. The present study was undertaken to identify the amount and distribution of progenitors like intermediate hepatocytes (IHs) and extraportal ductules (EPDs) in hepatic vasculopathies, and to see whether they bear any relation with GS expression patterns.

Methods: Forty six cases including Budd-Chiari syndrome [BCS] (17), acute hemorrhagic necrosis [AHN] (4), sinusoidal obstruction syndrome [SOS] (1), non-cirrhotic portal fibrosis [NCPF] (12) and extrahepatic portal vein obstruction [EHPVO] (12) were selected for K7, K19 and GS immunostaining. Mean ductular reaction (DR), IHs and EPDs were graded and zonation was recorded. GS staining pattern was recorded as absent, patchy and altered, normal, exaggerated.

Results: DR was seen most frequently in AHN and BCS with hemorrhage, and least in EHPVO. AHN and BCS with hemorrhage showed a trend towards increased K7 positive IHs, which correlated with absent or reduced GS staining, with loss of normal pattern. EPDs were also more common in AHN and BCS with hemorrhage, and showed a centrilobular pattern. Cases of NCPF showed variable IHs, EPDs and GS immunostaining. Cases of EHPVO showed predominantly a normal perivenular GS expression, with minimal IHs and EPDs.

Conclusions: Acute hepatic parenchymal loss as seen in AHN and BCS with hemorrhage activates IHs and EPDs, with loss of normal GS expression in early regenerative phase. Evaluation of IHs and EPDs, and correlation with GS expression in hepatic vasculopathies may provide novel insights in progenitor repopulation and prognosis.

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PIIINP is a useful marker of disease activity in hypereosinophilic syndrome with hepatic involvement

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Background: Hypereosinophilic syndrome (HES) is a rare disorder marked by peripheral eosinophilia and end organ dysfunction. In HES, hepatic involvement is uncommon and usually observed as multiple, small, hypoattenuated masses and most conspicuous during portal phase on contrasted computed tomography (CT). Increased level of type III procollagen-N-peptide (PIIINP) has been reported in certain systemic inflammatory conditions, but there is no article discussing PIIINP level in HES.

Case: We describe Japanese male patient with HES presenting hepatomegaly and geographic hepatic lesions which became more obvious in portal phase CT imaging. The patient was treated with prednisolone 60 mg daily as a starting dose and had a complete resolution of hepatic lesions in 9 months. The serum concentration of PIIINP was initially elevated and normalized in 2 months with treatment.

Result and discussion: Recent studies describe suppression of inflammatory activity improves degree of tissue fibrosis, and some reports stated level of PIIINP reflects severity of systemic inflammation. Based on these above, normalization of PIIINP by corticosteroid treatment suggests resolution of systemic inflammation as well as systemic fibrosis. Several literatures have described hepatic

geographic lesions and characteristic transient attenuation in HES. These findings are considered to be due to profound eosinophilic infiltration in the periportal area of the liver.

Conclusion: PIIINP may be a valuable serum marker to estimate disease activity in HES. For detecting hepatic involvement in HES, portal venous phase imaging on CT is the most effective.

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Comparison of fibroscan with MRI R2* to predict liver iron overload in thalassemia major

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Aim: We undertook this study to establish whether fibroscan can be used as an alternative to MRI R2* to predict the degree of iron overload and in turn, the degree of liver fibrosis in thalassemia major patients.

Methods: We evaluated 154 patients with thalassemia major, chronically dependent on blood transfusion and on iron chelator therapy. All patients underwent routine blood investigations, serum ferritin and tissue elastography (Fibroscan®402, Echosens, Paris) within 1 month of MRI T2* of the liver.

Results: Of total 154 patients, 99(64 %) were male. Mean age of patients was 12 ± 3.6 years (mean ± standard deviation (SD)). Total serum bilirubin, SGPT, SGOT and Serum albumin were 1.4 ± 0.6 mg/dl, 65.0 ± 51.8 IU/L, 62.9 ± 44 IU/L and 4.2 ± 0.2 g/d respectively. Mean fibroscan, MRI T2*(3T), corresponding MRI R2*(3T) and ferritin values were 8.2 ± 4.4 kPa, 3.18 ± 2.6 ms, 617.3 ± 549 Hz and 4712 ± 3301 ng/ml respectively. According to MRI, 67 (43.5 %), 49 (31.8 %) and 49 (14.3 %) patients had mild, moderate and severe iron overload, respectively. A strong correlation was found between fibroscan values and MRI R2* values (r = 0.85, p < 0.001). AUROC for fibroscan to detect severe, moderate and mild iron overload was 94.8, 84.5 and 84.7 %, respectively. Corresponding cut-offs of fibroscan values (kPa) to detect mild, moderate and severe iron overload were 5.5, 7.8 and 13.5, respectively. No correlation was found between fibroscan and ferritin (r = 0.19, p = 0.11).

Conclusion: Fibroscan can be used as an alternative to MRI R2* for predicting liver iron overload, in chronic transfusion dependent thalassemia major patients.

O-285

Adverse outcomes after non-hepatic surgery in patients with liver cirrhosis: a nationwide study

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Background: Postoperative adverse outcomes in patients with liver cirrhosis are not completely understood. This study evaluated the association between liver cirrhosis and adverse outcomes after non-hepatic surgery.

Methods: Reimbursement claims were used to identify patients with preoperative liver cirrhosis who underwent non-hepatic surgery from 2004 to 2007. Control patients without cirrhosis were matched by age,

sex, type of surgery and anesthesia. The adjusted odds ratios (ORs) and 95 % confidence intervals (CIs) of postoperative adverse events associated with liver cirrhosis were analyzed by multivariable logistic regression.

Results: Thirty-day mortality rates among 24,282 patients with cirrhosis and 97,128 control patients were 1.2 % (299 deaths) and 0.7 % (635 deaths) respectively. Liver cirrhosis was associated with postoperative 30-day mortality (OR 1.88, 95 % CI 1.63–2.16), acute renal failure (OR 1.52, 95 % CI 1.34–1.74), septicemia (OR 1.42, 1.33–1.51) and intensive care unit admission (OR 1.39, 95 % CI 1.33–1.45). Postoperative mortality increased in patients who had liver cirrhosis with viral hepatitis (OR 2.87, 95 % CI 1.55–5.30), alcohol dependence syndrome (OR 3.74, 2.64–5.31), jaundice (OR 5.47, 95 % CI 3.77–7.93), ascites (OR 5.85, 95 % CI 4.62–7.41), gastrointestinal hemorrhage (OR 3.01, 95 % CI 2.33–3.90) and hepatic coma (OR 5.11, 95 % CI 3.79–6.87).

Conclusion: Patients with liver cirrhosis had increased mortality and complications after non-hepatic surgery, particularly those with cirrhosis-related clinical indicators.

O-286

Clinical trial for regenerative therapy of cirrhosis using adipose tissue-derived stromal/stem cells

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Adipose tissue contains substantial number of pluripotent mesenchymal stem cells. We conducted the clinical trial for liver regenerative therapy of cirrhosis by intrahepatic arterial administration of freshly isolated autologous adipose tissue derived stromal/stem (regenerative) cells (ADRCs).

Methods: The objectives were cirrhosis patients who provided the written informed consent. The patients underwent liposuction of their subcutaneous adipose tissues in their abdomen or buttock. The obtained adipose tissues were immediately processed using the adipose-tissue dissociation equipment (Celution, Cytori Therapeutics Inc.) to obtain autologous ADRCs. The designated number of cells [$3.3 \times 10^5/\text{kg}$ ($n = 2$), $6.6 \times 10^5/\text{kg}$ ($n = 2$)] were infused through the catheter positioned at the hepatic artery. Safety evaluation was assessed 1 month after the treatment. Surface antigen of ADRCs and cultured cells (ADSCs) were assessed by FACS. Serum cytokine/chemokine concentration was measured by Bio-Plex.

Result: Four liver cirrhosis patients were enrolled with written informed consents (HI-01 (type C), HI-03 (type C), HI-04 (NASH), HI-05 (type B)). The number of infused ADRCs were 2.2×10^7 – 4.4×10^7 . Among ADRCs and ADSCs, 10.3–45.8 % and 80.9–98.9 % of cells, respectively, expressed the mesenchymal stem cell surface marker CD44. No severe adverse events occurred. Three among 4 treated patients improved serum albumin concentration during 3–6 months after treatment. Serum HGF, M-CSF, MIF, IL-18, and IL-6 were elevated in all 4 patients one day after treatment.

Conclusion: Intrahepatic arterial administration of autologous ADRCs were confirmed to be safely conducted without serious adverse events, and 3 patients increased serum albumin concentration after treatment.

O-287

Hepatic vein waveforms are independent of liver fibrosis in chronic hepatitis C patients

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Background: Hepatic vein waveforms in chronic liver disease is still a matter of debate. It's value in diagnosis of liver cirrhosis is disputed. It is unclear if there is any direct relationship between liver fibrosis and hepatic vein waveforms. We aimed to see the changes in hepatic vein waveforms in chronic hepatitis patients after attaining sustained virological response (SVR) after 24 weeks of successful treatment assuming that if waveform is fibrosan dependent, there would certainly be change in waveforms.

Methods: Consecutive chronic hepatitis C patients eligible for interferon ribavirin therapy treatment were enrolled from 2014 January. Those patients who attended SVR were subjected to fibrosan and hepatic vein waveforms measurements. Any changes in both parameters were analyzed.

Result: Seventy six patients were enrolled, 59 patients completed the treatment and 50 had attained SVR. There was male preponderance. Age ranged from 21 to 64 with mean 37.9 years. Predominant genotypes were 3 and 1. Pre-treatment mean liver stiffness was 10.48 ± 3.88 kPa. Waveforms seen were triphasic (76 %), biphasic (22 %) and flat (2 %). There was significant drop in stiffness after SVR. Out of 50 subjects, six subjects had change in waveform. There were significant association between Platelet count, spleen size and change in waveform. No significant association between Fibrosan with change in waveforms was found.

Conclusion: This study shows that there is no direct correlation of fibrosan value and hepatic vein waveform as even after decrease in liver stiffness after SVR, there was insignificant change in hepatic waveforms.

Poster Presentation

P-0001

Artificial peptides derived hepatitis c virus core protein interfere virus replication in vitro

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Objective: The combination therapy for chronic hepatitis C prevents the drug of resistant mutants. New drug and targets still are demanded. We designed some artificial peptides and assessed the ability of them interfering virus replication.

Methods: The artificial peptides contained the functional domain derived hepatitis c virus core protein, penetrating peptide and localization signal sequence. The peptides had different sub-cellular localization, such as the endoplasmic reticulum and the lipid droplets. We determined the effect of these peptides on the different genotype HCV replication in vitro.

Results: we found some peptides which interfered with the interaction of HCV core protein and other proteins, reduced progeny virus. They inhibited HCV replication independent of virus genotype.

Conclusion: HCV core protein is an important target for anti-virus drug. Peptide derived from HCV core protein can inhibit virus replication.

P-0002

Amino acid substitutions in ISDR of HCV-NS5A affect infectious virus production and ISG induction

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Background and aim: The number of amino acid substitutions in IFN sensitivity-determining region (ISDR) is known to be a potent predictor for outcome of IFN-based therapy. Although several clinical studies have already proven this association, the underlying mechanism of ISDR is still underestimation. In this study, we aimed to elucidate the effect of these substitutions in ISDR on HCV life cycle and IFN sensitivity by use of JFH-1 based cell culture system.

Methods: Because the clinical studies of ISDR were mainly executed in patients with HCV genotype 1b, we used the recombinant JFH-1 virus replaced with NS5A of genotype 1b Con1 strain (JFH-1/5ACon1). We also used another recombinant virus introduced 7 amino acid substitutions into JFH-1/5ACon1 (JFH1/5ACon1/i-7mut), and compared the virus propagation and IFN susceptibility.

Results: We found that the infectious virus production was attenuated in JFH1/5ACon1/i-7mut as compared with JFH1/5ACon1, although the replication level was comparable. By administration of IFN, the difference of susceptibility was not detected. By transfecting the full-length RNA, mRNAs of ISGs were sufficiently induced in JFH1/5ACon1/i-7mut transfected cells, but not in JFH1/5ACon1 transfected. These data suggested that the inhibition of ISGs induction by NS5A with wild-type ISDR was disrupted by 7 amino acid substitutions.

Conclusion: By use of recombinant JFH-1 viruses with NS5A of genotype 1b strains, we could demonstrate that amino acid substitutions in ISDR were associated with the infectious virus production and ISGs induction.

P-0003

Association between microRNA Let-7g and hepatitis C

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Aim: MicroRNAs (miRNAs) are known to regulate of HCV infection and host cellular development. We aimed to evaluate the interaction between the let-7g and hepatitis C virus (HCV) infection.

Methods: We used the replicon cells including Huh7/Ava.5, Huh7/J6/JFH and Huh7.5/Con 1 for HCV genotype 1b and 2a. Expression levels of let-7g were determined by using the quantitative real-time PCR. The 1.0 and 0.5-kb fragments of the let-7g promoter were amplified by PCR. Site-directed mutagenesis of the AP-1 binding-site on promoter region of the let-7g was performed. Liver tissues of patients with HCV genotype 1 infection and non-alcoholic fatty liver disease are used to compare the let-7g expression.

Aim: MicroRNAs (miRNAs) are known to regulate of HCV infection and host cellular development. We aimed to evaluate the interaction between the let-7g and hepatitis C virus (HCV) infection.

Results: In the five hepatic cell lines, the let-7g expression is lower than let-7b expression. We also found the effects of let-7g have similarity to let-7b on HCV RNA and NS3 protein level and RNA viral load. Let-7g was found to be able to target 5'-UTR of HCV genome. The interferon/ribavirin treatment increased let-7g expression in Ava.5 cells and Huh7 cells. After we deleted nine nucleotides of the putative activator protein-1 (AP-1) binding site present in the let-7g promoter, interferon/ribavirin -induced let-7g promoter activity was decreased in Ava.5 cells. In patients with chronic hepatitis C with HCV genotype 1 infection, the let-7g is significantly decreased when compared to patients with non-alcoholic fatty liver disease.

Conclusions: There is an important role of let-7 g on the HCV infection. Interferon/ribavirin /ribavirin induces let-7g expression through AP-1 binding site on the promoter of the let-7g.

P-0004

Combined HRM with Bayes discriminant method: a novel HCV genotyping method

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Objective: To observe the accuracy, sensitivity and cost of a novel HCV genotyping technique combined high resolution melting analysis (HRM) with Bayes discriminant method.

Methods: Target gene fragment which include 5'-untranslated and core region was selected. Four or five inner amplicons were amplified with nested PCR technique, followed by HRM analysis and determined their melting temperature (Tm). Finally, HCV genotypes were classified by Bayes discriminant method.

Results: 101 samples obtained genotyping results in 108 HCV samples whose genotypes were unknown. 99 samples were correctly classified (HCV genotypes were classified into 1b, 3a, 3b, 2a–6a). Both the overall accurate rate and cross validation accurate rate were 98.0 %. While the accurate rate of 1b, 2a–6a, 3a, and 3b was 100, 96.9, 95.5, and 100 % respectively. In supplementary genotyping experiment for 2a–6a, all of 32 samples were classified correctly. Comparing with type-specific probe technique, difference of accurate rate was not significant between HRM genotyping and the former (98.0 vs 100 %, $p = 0.503$). However, limit detection (5×10^3 vs 5×10^4 copies / mL) and cost (54.6 /60.0 vs 100 per sample) of HRM were lower. Comparing with direct sequencing, limit detection of HRM was the same as the former, but cost was lower (54.6 /60.0 vs 120 per sample). **Conclusions:** Compared with type-specific probe and sequencing technique, this novel method equipped with high accuracy, high sensitivity and low cost so that it would be introduced as a more perfect HCV genotyping technique.

P-0005

Host factors for HCV replication and infectious virus production in Vero cells**Asako Murayama, Nao Sugiyama, Takaji Wakita, Takanobu Kato**

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Hepatitis C virus (HCV) cell culture system with JFH-1 strain and a human hepatoma cell line, HuH-7, enabled us to produce infectious HCV particles. Several host factors associated with HCV life cycle have been identified and some of them were considered as the restriction factors for HCV replication in hepatocytes. By supplementation of these factors to non-hepatic cells, HCV production became possible in such cell lines. Vero cells established from the monkey's kidney are commonly used for production of vaccines against a variety of viruses. In this study, we aimed to establish the novel cell line susceptible for infectious HCV particle production. The authentic Vero cells were not susceptible for HCV infection or replication. The expression level of miR-122, one of the essential factors for HCV replication, is notably low in Vero cells. The supplementation of miR-122 enabled HCV replication in Vero cells. However, Vero cells expressing miR-122 were not permissive for HCV infection. We supplemented Vero cells with the HCV receptor molecules and found that the SR-BI was the essential factor for HCV infection in Vero cells. The supplementation of Apolipoprotein E (ApoE), one of the host factors important for virus production, enabled infectious virus production in Vero cells. Finally, we established Vero cells expressing miR-122, SR-BI and ApoE. In these cells, HCV infection, replication and infectious virus production could be observed. In conclusion, we demonstrate that miR-122, SR-BI and ApoE are necessary and sufficient for the reconstitution of the complete HCV life cycle in non-human non-hepatic Vero cells.

P-0006

Hypermethylation of placental bikunin gene in chronic hepatitis C virus infection**Ragaa A. Ramadan, Wafaa S. Ragab, Moyassar A. Zaki, Ahmed M. Awad**

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Aim: Epigenetics are heritable states of genetic expression caused by changes in chromatin structure without variations in DNA sequence, thus explaining how a single genotype can lead to multiple phenotypes. Hepatic stellate cells differentiation from the quiescent to the fibrogenic phenotype has been linked to epigenetic changes namely DNA methylation and chromatin remodelling. Studies reported increased methylation of genes such as Rasal1 and Thy1 along with fibrosis progression. Bikunin gene encodes a serine protease inhibitor which inhibits the formation of active hepatocyte growth factor that is considered a potent mitogen for hepatocytes with liver regeneration effects. We investigated the methylation status of promoter region of bikunin gene in chronic hepatitis C virus (HCV) infected patients and its relation to disease course.

Methods: Methyl specific polymerase chain reaction of bikunin gene was carried for 50 healthy adults and 50 HCV patients whom were sorted according to Child-Pugh (CP) scoring system into 46 % in class A, 42 % in class B and 12 % in class C. There was a significant increase in methylation from 40 % in control to 64 % in patients

($p = 0.01$), but no correlation was found with clinicopathological criteria such as CP score or HCV viral load.

Conclusion: This underpins methylation changes induced by HCV, hence the role of demethylating agents should be the focus of future research.

P-0007

Non-neutralizing epitopes induce robust ADCC mediated by CD56⁺ NK cells in chronic HCV infection**Tao Shen¹, Lu Long¹, Manxue Jia², Hua Liang², Xiangbo Huang¹, Fengmin Lu¹**¹Department of Microbiology and Infectious Disease Center, School of Basic Medical Sciences, Peking University, Beijing, China;²National Center for AIDS/STD Control and Prevention, China CDC, Beijing, China

Background: CD56⁺ NK cell-mediated antibody-dependent cellular cytotoxicity (NK-ADCC) is of considerable interest in viral infection. However, limited knowledge was known about NK-ADCC responses in chronic HCV infection.

Methods: In our study, NK cell-mediated ADCC were detected and compared between 29 chronic HCV carriers and 49 healthy individuals. The capacity of NK-ADCC supernatants to inhibit HCV replication in vitro was analyzed and HCV-specific ADCC epitopes with capacity to induce robust NK-ADCC activation were identified.

Results: Our results indicated that impaired NK-ADCC function was observed in chronic HCV infection, reflecting by decreased degranulation (CD107a) and IFN-gamma production of NK cells triggered by recognition of CD16 to antibody-bound p815 cells. CD56^{dim} NK cells subsets mainly attributed to the NK-ADCC activation. In vitro higher NK-ADCC responses were associated with abnormal serum ALT (>40 IU/ml) in HCV carriers. Additionally, NK-ADCC supernatants from chronic HCV carriers had defective capacity to inhibit HCV replication in vitro. HCV-E1/E2 Ab-recognized peptide pool induced NK-ADCC responses in sera from approximate half of chronic HCV carriers. Finally, five linear NK-ADCC epitopes (aa211-aa217, aa384-aa391, aa464-aa475, aa544-aa551 and aa648-aa659) on HCV envelope were identified and shown no overlap with the putative linear neutralizing epitopes.

Conclusions: This study revealed the dysfunctional characteristics of NK-mediated ADCC responses in chronic HCV carrier. The key non-neutralizing NK-ADCC epitopes identified in our study may act as new targets for immunologic intervention.

P-0008

NKG2D, the mediator of host T cell response, correlates to naturally occurring HCV NS5A variants**Po-sung Chu¹, Aya Ugamura¹, Shingo Usui^{1,2}, Hirotochi Ebinuma¹, Hedetsugu Saito^{1,3}, Takanori Kanai¹**¹Department of Gastroenterology and Hepatology, School of Medicine, Keio University, Tokyo, Japan; ²National Saitama Hospital, Saitama, Japan; ³Division of Pharmacotherapeutics, School of Pharmacy, Keio University, Tokyo, Japan

Background and aims: T-cell responses play a central role in the natural history and pathogenesis of viral hepatitis. HCV is known to escape from T cell mediated responses by mutation in cognate epitopes (Bowen DG, et al. JEM 2005). Differential NKG2D expression

of CD56 positive T cells between genotypes of HCV infections predicts rapid and sustained viral responses to interferon-based therapies (Chu PS et al. PLoS One 2015). Interferon-free direct-acting antiviral agents targeting viral proteins including NS5A have been the recommended for treatment to genotype 1 HCV in the US, EU and in Japan. However, whether specific host immunity causes the selection of variant quasi-species is still unclear.

Methods: With prior informed consents, peripheral blood mononuclear cells (PBMCs) were isolated from patients of genotype 1 chronic hepatitis C and then were analyzed with flowcytometry. Amino acid substitutions were determined by direct sequencing of viral PCR.

Results: Of the 100 patients, 15 patients were found to have substitution with Y93H (15 %) and 2 with L31 M (2 %). Patients with Y93H substitution have relatively lower serum ALT and higher HCV-RNA. Peripheral blood NKG2D expressing cells from patients with Y93H substitution show significantly higher expression of NKG2D in NK cell ($p = 0.0064$), NKT cells ($p = 0.03$), and cytotoxic T cells ($p = 0.0475$).

Conclusion: Higher peripheral blood NKG2D expression correlates significantly with the existence of Y93H substitution, a known resistance associated variants (RAVs). Since higher NKG2D expression correlates SVR to interferon-based therapies, patients with Y93H substitution may benefit from interferon-based therapy.

P-0009

Primary hepatocyte of *Tupaia javanica* as a model for hepatitis C virus infection

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Study of HCV infection has been hampered by the lack of an *in vitro* or *in vivo* small animal model. Recently, tree shrew *Tupaia belangeri* has been shown to be susceptible to infection with Hepatitis viruses, however this animal distribution in the wild is limited to its habitat in China. This research aimed to investigate whether primary tupaia hepatocytes (PTH) from *Tupaia javanica* commonly found in west part of Indonesia are susceptible to HCV infection using archived HCV-positive patient sample. Primary tupaia hepatocytes were infected with HCV by overnight incubation with HCV-positive plasma samples. HCV-negative plasma sample was used as control. At day 0, 1, 3, 5, 7 and 15 after infection, culture medium and hepatocyte cells were collected and analyzed. Nested RT-PCR was used to detect HCV RNA in culture medium. HCV replication was analyzed using rTth RT-PCR and quantitative analysis was performed using commercial real time RT-PCR kit. HCV RNA was detected in culture medium from day 0 to day 15, whilst in PTH, positive- and negative-strand HCV RNA were also detected. However, HCV RNA quantitation could not be determined due to limited number of hepatocyte cells. In conclusion, the results suggest that *Tupaia javanica* has shown susceptibility to HCV infection and can then be considered as a possible candidate for a small experimental animal model for HCV study.

P-0010

Extracellular matrices impair the IFN- α signaling via the beta 1 integrin-mediated signaling

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The interferon alpha (IFN α) based treatment for chronic hepatitis C patients have been widely carried out all over the world. Among them, we often experienced treatment-resistance in patients with advanced liver fibrosis. So we investigated the reason why the liver fibrosis affecting the interferon signaling in the molecular level. Human hepatoma cell line Huh7 and OR6 (Huh7 stably harboring full-length HCV replicon) were cultured on extracellular matrix (ECM) coated dishes (CD) or non-coated plastic dishes (ND), and treated with human IFN α . After IFN α treatment, luciferase activities, virus proteins or RNAs, ISG proteins or ISG RNAs were compared between cells cultured on CD and ND. Blocking antibody against beta1 (b1) integrin, inhibitors against focal adhesion kinase (FAK) and integrin-linked kinase (ILK) were used. In Huh7 cultured on CD, ISRE luciferase activity was significantly lower than in cells on ND. ISG protein expressions were also lower. In Huh7 cultured on CD, the production of ISG proteins and the ISRE activity was limited. Amount of HCV-RNA from OR6 cultured on CD after IFN- α treatment was higher in comparison of that from cells on ND. Similar results were shown in HCV protein expression. The effect of IFN α against to HCV replication was also suppressed in cells on CD. When cells were treated with b1 integrin blocking antibody, ILK or FAK inhibitor, ISRE luciferase activity was restored and ISG expression was increased while the expression of HCV proteins was suppressed. These results suggested that b1 integrin-mediated signals could affect the interferon signaling.

P-0011

Inhibitory effect of miR-125b on HCV core protein-induced TLR2/MyD88 signaling in THP-1 cells

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Objectives: Macrophages/Monocytes have been shown to be important immune cells mediating both innate and adaptive immunity in hepatitis C virus (HCV) infected patients. Many anti-pathogen pathways, including pattern recognition Toll-like receptors (TLRs) mediating signaling, are known to be regulated by a network of microRNAs. In this study, we investigated the possible role of miR-125b in regulating monocyte immune responses induced by HCV core protein.

Methods: Monocytic THP-1 cells were treated with various concentrations of recombinant HCV core protein. Expression of cytokines and miR-125b in these cells were analyzed. The requirement of TLR2 or MyD88 genes in HCV core protein induced immune responses was determined by transfection of THP-1 with gene

knockdown vectors expressing either TLR2 siRNA or MyD88 siRNA. The effect of miR-125b overexpression on TLR2/MyD88 signaling was performed by transfection cells with miR-125b Mimic RNA oligos.

Results: Responding to HCV core protein stimulation, cytokine production was up-regulated and miR-125b expression was down-regulated in THP-1 cells. The modulatory effect of HCV core protein on cellular events was dose- dependent; and required functional TLR2 or MyD88 genes. Forced expression of miR-125b abolished HCV core protein induced cellular responses by inhibiting MyD88 mediated signaling, including phosphorylation of NF- κ Bp65, ERK and P38.

Conclusions: The inverse relationship between expressions of miR-125b and cytokine after HCV core protein challenge suggests that miR-125b may negatively regulate HCV induced host immune responses through targeting TLR2/MyD88 signaling in monocytes.

P-0012

A study on distribution of HCV genotypes in South India

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Background and objectives: Hepatitis C virus is one of the leading cause of chronic hepatitis, cirrhosis and hepatocellular carcinoma worldwide. Hepatitis C is divided into 6 distinct genotypes with multiple subtypes worldwide. Identification of genotype is important in designing the therapeutic strategies. This study was undertaken to find out the prevalence of most common genotype in South India.

Methods: 35 consecutive new patients with hepatitis C who came to our OPD from october 2013 to september 2015 were enrolled in this study. HCV RNA Quantification was done by cobas taqman method and HCV genotyping were determined by nested PCR.

Materials: Total number of patients enrolled is 35. 22 patients were males and 13 were females. History of blood transfusions were present in 18 patients (51.42 %). Out of these 35 patients, 22 patients had Genotype 1 (62.8 %). In that 22 patients the most common subtype is 1a which is present in 16 patients (72.7 %). 8 patients had Genotype 3 (22.8 %), 3 patients had Genotype 2 (8.5 %) and 2 patients had genotype 4 (5.7 %). HCV RNA was found to be very high in Genotype 1 compared to other Genotypes.

Conclusions: There were various case reports suggesting that genotype 3 is the most prevalent genotype in india. But in our study Genotype 1 accounts for almost 62 % of patients and patients with Genotype 1 has high viral load compared to other genotypes in our part of India.

P-0013

Epidemiologic features in Chinese descent in Burmese with CHC in Hospital of Dehong District, China

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Objective: To analyze and compare the epidemiologic and clinical data between Chinese descent in Burmese and Chinese, China. To understand the features of CHC in Myanmar.

Methods: 172 patients including Chinese descent and Chinese enrolled from 2009 to 2012 were collected. We analyzed the character of CHC in Myanmar.

Results: (1) They composed of mainly Chinese descent. The Chinese descent were mostly consisted of female, and the average age of them were more than 40. The occupations of most Burmese were merchants. Burmese were infected mainly by medical pathway. (2) The clinic feature for most of Burmese was that HCV was found accidentally. The courses for most of Burmese were less than 10 years. And patients with HIV-HCV were less than Chinese. The HCV RNA level, the portion of patients with LC and the average course of diseases in the Burmese had no statistically significant differences compared to that in the Chinese. And the genotypes were mainly genotype 3b, 6 and other ones which could't be detected.

Conclusion: (1) The Chinese descent who were infected mainly by medical pathway were mainly female and more than 40 years old and most of them were merchants. The portion of patients with liver cirrhosis and the course had no statistically significant differences between Burmese patients and Chinese ones. (2) The genotypes of HCV in Myanmar were diversity and they were mainly genotype 3, 6 and ones that could't be detected. The portion of Chinese descent patients with HCV/HIV was lower than that of Chinese ones.

P-0014

Decreasing hepatitis C virus infection in Thailand in the past decade; an evidence-based study

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Hepatitis C virus (HCV) infection affects more than 180 million individuals worldwide. Recent advances in direct-acting therapeutics promise effective treatments for chronic HCV, but only if the affected individuals are identified. Therefore, treatment coverage requires accurate epidemiological data about HCV infection. This study, we performed molecular surveillance to determine the current prevalence of chronic HCV carriers throughout Thailand, which in 2004 was estimated to have a significant number of HCV carriers. In total, 5964 serum samples from Thai residents (6 months to 71 years old) were obtained in 2014 and screened for the anti-HCV antibody. Positive samples were further analyzed using RT-PCR, sequencing, and phylogenetic analyses. Although the lowest HCV seropositivity rate in the southern region (0.58 %) differed slightly from that found in the northeast (1.22 %), there were no significant differences in seroprevalence among the 4 regions of Thailand ($p = 0.340$). The

seropositivity rates increase with age and highest in individuals who were 41–50 years old. These results suggest that approximately 759,000 individuals are anti-HCV-positive and 357,000 individuals currently have viremia HCV infection. These numbers represent a significant decline in the prevalence of HCV infection found and reported a decade ago. Interestingly, the frequency of the genotype 6 increased from 8.9 to 34.8 %, while genotype 1b declined by half (from 27 to 13 %). These most recent estimates of HCV burden in Thailand provide valuable evidence-based awareness that could be used to administer priority treatments to the affected population groups, cost-effectively allocate resources, and improve national public health policy.

P-0015

Determining modes of transmission of hepatitis C virus (HCV)

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Introduction: With the availability of new highly effective drugs against HCV, treatment goals of HCV seem achievable, yet complete prevention of HCV transmission is still a distant objective. This study was planned to determine the significance of various factors in HCV transmission.

Subjects and methods: 200 patients with HCV and 500 blood donors were subjected to interview regarding risk factors pertaining to high risk of HCV transmission such as blood transmission, dialysis, circumcision, tattooing, body piercing, shaving habit, surgery and multiple injections.

Results: HCV patients (mean age: 49.2 ± 13.74 years) were older as compared to blood donors (35.13 ± 9.80). 44 % of HCV individuals were females, as against 20.80 % of Blood donors. None of our patients had a history of injection drug abuse. On regression analysis, history of blood transfusion ($p < 0.001$, OR 96.16), dialysis ($p < 0.001$, OR 36.19), circumcision ($p < 0.001$, OR 11.75), shaving in a barber shop (0.002, OR 3.34), body piercing ($p = 0.002$, OR 4.40) and a history of multiple injections (< 0.001 , OR 5.98) were the factors found significantly associated with HCV population. Tattooing ($p = 0.071$, OR 0.10) and dental treatment ($p = 0.006$, OR 0.20) were more prevalent in blood donors. Average number of injections received for common medical ailments per patient/year was 5.53 ± 4.75 in HCV group (0.74 ± 2.48 in blood donors).

Conclusion: With advances in blood transfusion practices and decrease in HCV transmission via this route, other percutaneous routes including unsafe injection practices play a significant risk in HCV transmission.

P-0016

Distribution of hepatitis C virus genotypes in injection drug users with chronic hepatitis C

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Aim: To determine the distribution of various genotypes of hepatitis C virus (HCV) in injection drug users (IDUs) with chronic HCV infection.

Method: After the HCV-RNA isolation (MagNA Pure LC Total Nucleic Acid Isolation Kit, Roche), viral load was determined according to COBAS TaqMan 48 (Roche Diagnostic, ABD) procedure. This was performed with real time polymerase chain reaction (RT-PCR) technique. HCV genotypes were studied by AMPLIQUITY HCV-TS (AB Analitica) kits.

Results: Genotype 1 was observed in 120 of the 238 IDUs (50.4 %) with chronic HCV infection. Of these, 112 IDUs showed infection with subtype 1a (93.3 %) and 8 with subtype 1b (6.7 %). Genotype 3 was determined in 81 IDUs (34 % of all cases), genotype 2 in 22 (9.3 % of all cases), and genotype 4 in 15 IDUs (6.3 % of all cases). Genotypes 5 and 6 were not found in the study population. In this study we found that there was no association of HCV genotype with patient age and serum HCV RNA levels.

Conclusion: There is a correlation between certain groups and predominant HCV genotypes. For example, 3a and 1b are the prevailing genotypes in IDUs of western countries whereas genotype 4 is responsible for most of infections in IDUs of North-eastern of Poland. In our country the number of studies concerning IDUs with chronic hepatitis C is very scanty. Reaching and managing IDUs with chronic hepatitis C infection is difficult. This study demonstrated that genotype 1a and genotype 3 are predominant genotypes in our troublesome population.

P-0017

HCV genotype-specific epitope peptides screen and B-cell epitope prediction

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Aim: Epitope polypeptides generated based on conserved sequences assessment and cell epitope prediction, become hot spot of science research nowadays, because of its high conservation and strong antigenicity.

Method: 4 HCV B-cell epitope polypeptides were constructed based on conserved sequences assessment in HCV E2 region and B-cell epitope prediction, including genotype 1a, 1b, 4d and concomitant sequences respectively. OD450 values were assayed by ELISA, when epitope polypeptides were combined with serum from 29 HCV infection and 25 healthy controls. Differences were analyzed by SPSS v20.0 software. T/T' test methods were used for comparisons between two groups. By these, we could confirm the differences and statistical significance between HCV infection group and control group.

Result: Serous associativities of genotype 1a, 4d specific and concomitant epitope polypeptides with HCV infection patients were higher than none-HCV infection people and serous associativity of genotype 1b specific epitope polypeptides with HCV 1b infection patients was higher than HCV 2a infection patients. HCV genotype-specific epitope polypeptides were screened preliminary.

Conclusion: We established HCV and genotype serological ELISA detection technology; Screened HCV genotype-specific epitope peptides based on conservation assessment and B-cell epitope prediction in HCV E2 region.

P-0018

Interfamilial transmission of hepatitis C genotype 4 in Egypt**Mohamed Elateek¹, Eman Eissa², Hesan Khalaf¹, Ibrahim Rabea¹, R. Mohamed²**¹Tanta Hepatology Research Centre, Tanta, Egypt; ²Banha Teaching Hospital, Banha, Egypt**Introduction:** As the prevalence rate in Egypt about 14.8 % and the incidence rate not less than 150,000 new cases annually (demographic study 2008) and although of start of DAA therapy in Egyptian project for control of virus hepatitis, the role of prevention is very important in control the virus so we look for the possible mode of transmutation.**Patients and methods:** This study was conducted in Tanta hepatology research center on 640 adult patients under the treatment by DAA, family history was done and investigations were done freely for adult members in these families who give history of previous HCV antibody positivity and who is positive PCR start treatment freely (governmental support) if he is suitable for treatment.**Results:** In 640 family, we found 230 one member more than the original one infected with virus c (36 %)-131 were female (56.9 %)-99 were male (43 %)-age from 20–63 years old; average 39 years-120 from 640 were spouses (18.7 %) and the other were parents or offspring-20 family had more than 2 infected member (3.1 %) rather than the original member.**Conclusion:** In community maximum prevalence 14.8 %—significant increase in interfamilial transmission in this study (36 %)—from spouses significant increase in prevalence (18.7) than the international sexual transmission prevalence (6 %).**Recommendation:** Interfamilial transmission and sexual transmission of virus C need more studies and may change the strategy of control and prevention.

P-0019

Elderly and low serum 25(OH) D3 level contributes to drug resistance of NS5A region**Tomomi Okubo¹, Masanori Atsukawa¹, Noritomo Shimada², Hiroshi Abe³, Taegang Arai¹, Ai Nakagawa¹, Norio Itokawa¹, Chisa Kondo¹, Yoshio Aizawa³, Katsuhiko Iwakiri⁴**¹Nippon Medical School Chiba Hokusoh Hospital, Chiba, Japan; ²Otakanomori Hospital, Kashiwa, Japan; ³The Jikei University Katsushika Medical Center, Tokyo, Japan; ⁴Nippon Medical School Hospital, Chiba, Japan**Purpose:** Current treatment strategies for chronic hepatitis C involve combination of various direct-acting antiviral agents (DAAs), with some of the key drugs being NS5A inhibitors. The purpose of this study is to clarify the characteristics of the baseline factors of patients with drug-resistance to DAAs at the NS5A region.**Methods:** Of 247 consecutive patients with genotype 1b, chronic hepatitis C who visited each institution between December 2014 and September 2015, the RAVs of L31 and Y93 at NS5A region were examined by a direct sequence method.**Results:** Patients consisted of a median age of 70 (24–87), 111 females (44.9 %). Median serum 25 (OH) D3 level were 20 ng/ml(6–64) and 153 patients have the IL28B TT genotype (rs8099917). L31 and Y93 variants at the NS5A region were detected in 3.7 % (9/247) and 13.4 % (33/247) of patients, respectively. Patients with the L31 variant had no significant characteristics. Univariate analysis identified factors contributed to Y93 variant as high level of HCV RNA, IL28B TT genotype, serum 25(OH) D3 deficiency, and higher age. In multivariate analysis, serum 25 (OH) D3 deficiency ($p = 0.0012$, $OR = 3.8457$) and higher age ($p = 0.0282$, $OR = 1.0478$) were identified independent factors. Using ROC curve analysis, the cutoff value having Y93 variant was 20 ng/mL. The patients with 25(OH)D3 of <20 ng/mL have frequently Y93 variants compared to those of 25(OH)D3 of >20 ng/mL [$p = 0.005$, 25.9 % (29/112) vs 8.9 % (12/135)].**Conclusion:** In the present study, elderly and patients with deficiency of serum 25 (OH) D3 have frequently RAVs at NS5A region.

P-0020

Elimination of hepatitis C virus (HCV) in South Korea**Do Young Kim¹, Young-Suk Lim², Moon Seok Choi³, Young Seok Kim⁴, Sarah Blach⁵, Homie Razavi⁵, Kwang-Hyub Han¹**¹Department of Internal Medicine, Yonsei University College of Medicine, Seoul, Korea; ²Department of Gastroenterology, Asan Medical Center, University of Ulsan College of Medicine, Songpa-gu, Seoul, Korea; ³Department of Medicine, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea; ⁴Department of Internal Medicine, Soonchunhyang University Bucheon Hospital, Bucheon, Korea; ⁵Center for Disease Analysis (CDA), Lafayette, CO, USA**Background:** In South Korea, the anti-hepatitis C virus (HCV) prevalence is ~1.2 % among adults. This study sought to quantify the burden of HCV and identify strategies to eliminate HCV infections by 2030.**Methods:** Using a Markov model, the HCV infected population and future disease progression were forecasted. The impact of intervention strategies (prevention, treatment and screening) on projected disease burden was measured.**Results:** The average age of the HCV infected population is 55 years. Under today's treatment paradigm, all cause mortality, liver related deaths (LRD) and treatment (4500 patients/year) will result in a 40 % decrease in HCV infections by 2030. However, the number of patients with end stage liver diseases (ESLD) and LRD will increase 1–4 %. A prevention campaign to reduce new infections by 70 % from 2017–2025 would reduce total HCV infections by an additional 17 % (by 2030) with no impact on ESLD and LRD. Increasing the sustained viral response (SVR) to 90 % (treating 4500 patients/year) would reduce total infections by 16 % and ESLD and LRD by 25–30 %, by 2030. Eliminating HCV by 2030 requires combining the above interventions with increased treatment (16,300 patients/year by 2020). This will reduce total HCV infections by 92 % and ESLD and LRD by 75–85 % by 2030. After 2029, the annual number of treated patients would decline as there become fewer infections overall. This strategy requires expanded screening starting in 2017.**Conclusions:** Elimination of HCV infection within the next 15 years is feasible by combining prevention, higher SVR therapies, increased screening and treatment.

P-0021

Emergence of hepatitis C virus genotype recombinant forms 2 k/1b in Georgia

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Hepatitis C virus (HCV) evolution is thought to proceed by mutations within the six major genotypes. Studies of HCV recombinant genotypes in different parts of the world have been initiated recently. Only few cases are identified worldwide, predominantly in Eastern Europe. In 2011 we detected the recombinant form of HCV genotype (HCV-RF) 2 k/1b in Georgia. We analyzed data of HCV genotypes in our center for 2 years, in 491 patients with chronic hepatitis C virus infection. Initially all studies were performed on Siemens VERSANT HCV genotype assay (LiPA), a second generation (Limbach Laboratory, Heidelberg, Germany). Afterwards all HCV genotype 2a/2c were partly sequenced (NS5A region). The results were surprising: approximately 2/3 of genotype 2 and 19 % of all HCV genotypes appeared to be HCV-RF 2 k/1b. We can conclude that almost every 5th of HCV infected Georgian patient has HCV-RF 2 k/1b. High prevalence of recombinant genotype in our country warrants further analyses of available data.

P-0022

Frequency and distribution of discrepant anti-HCV screening results in Korea during recent 4 years

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Various automated analyzers for detection of antibody to hepatitis C virus (HCV) are commercially available in clinical laboratories, because anti-HCV testing is still the first step to screen and diagnose hepatitis C. Since anti-HCV assays were developed with different antigenic targets according to different manufacturers, interpretation of borderline results is uncertain and difficult. Herein, we report the frequency and distribution of discrepant anti-HCV screening results among three commonly used assays. Among a total of 152,688 serum samples analyzed during 4-years period (from March 2011 to September 2015), samples which were tested both by Elecsys anti-HCV assay (Roche Diagnostics, Germany) or Architect anti-HCV assay (Abbott Laboratories, IL, USA) as the first-line routine test and by Vitros anti-HCV assay (Ortho-Clinical Diagnostics, UK) as the back-up test were reviewed in this study. A total of 692 samples from 624 patients showed discrepant or borderline results by two or three assays with frequency of 0.5 %. Nineteen samples were tested by all three assays; 12 cases with only Architect showing positive result, 4 cases with only Elecsys showing negative result, 2 cases with only Vitros showing negative result and 1 case with only Vitros showing positive result. Elecsys and Architect tended to report positive results when compared with Vitros among discrepant outcomes (Table 1). In conclusion, we report the frequency and distribution of discrepant anti-HCV results among three assays. Our results would provide

insights to laboratory physicians in understanding the tendency of each analyzer when compared with other assays.

P-0023

Genetic variants in interferon-lambda 4 influences HCV clearance in Chinese Han population

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Background: Recent many studies indicated a novel dinucleotide variant in ss469415590 (TT vs. ΔG) of interferon-λ4 (IFNL4) gene strongly associated with hepatitis C virus clearance. To evaluate the impact and clinical usefulness of IFNL4 ss469415590 genotype on predicting both spontaneous HCV clearance and response to therapy in Chinese population.

Methods: We genotyped 795 chronic HCV carriers, 460 subjects with HCV natural clearance and 362 patients with pegylated interferon- α and ribavirin (PEG IFN- α /RBV) treatment.

Results: IFNL4 ss469415590 variant genotypes significantly decreased host HCV clearance, both spontaneous (dominant model: OR = 0.50, 95 % CI = 0.36–0.71) and INF- α induced (dominant model: OR = 0.32, 95 % CI = 0.18–0.56). Multivariate stepwise analysis indicated that ss469415590, rs12979860, the level of baseline HCV RNA and platelet were as independent predictors for sustained virological response (SVR). But the area under the ROC curve (AUC) was only 0.58 for ss469415590, and it was elevated to 0.71 by adding rs12979860, baseline HCV RNA and platelet in the prediction model of SVR.

Conclusions: These findings underscore that although genetic factors of host and pathogen were commonly important during HCV clearance, ss469415590 may be also a strongly predictive marker in the Chinese population.

P-0024

Rising inpatient hospital charges (IHC) in ethnically diverse U.S. chronic hepatitis C (CHC) patients

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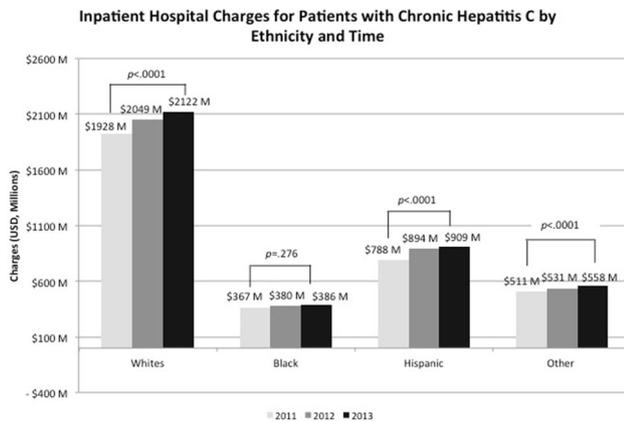
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Background: CHC affects 170 million people worldwide and is the leading cause of cirrhosis and hepatocellular carcinoma (HCC) in the US. Our aim is to assess IHC incurred in patients with CHC in California, the largest state with an ethnically diverse population of 38.8 million people.

Methods: De-identified data was obtained from the California Patient Discharge Database. ICD-9 diagnosis codes were used to identify records with either primary or secondary admitting diagnoses of CHC, resulting in 40,374 records with CHC for analysis. Charges were in US dollars (\$).

Results: Total charge for 2011–2013 was \$8.9 billion with the majority in male (66 %) and ages 45–64 (72 %). Of the total charge, Whites constituted 69 % followed by Hispanics (29 %), Other Ethnicities (18 %, inclusive of) Asian and Pacific Islanders and Blacks (13 %). Admissions relating to cirrhosis accounted for 41 % of the total charge and HCC 6 %. Medicare covered the majority of the cost at 35 %, followed by Medi-Cal (34 %), Other Coverage (16 %) and Private Insurance (14 %). Importantly, total charges also increased significantly from \$2.8 billion in 2011 to \$3.0 billion in 2012 to \$3.1 billion in 2013 ($p < 0.0001$). This increasing trend was also seen in sub-analysis by ethnicity, particularly in Whites, Hispanics and others group (Fig).

Conclusions: The economic burden of IHC in patients with CHC is high, suggesting significant resource utilization for hospital care in patients with CHC, and it is increasing across ethnicities. Early linkage to care and treatment may prevent morbidities requiring inpatient hospital care and their associated direct cost.



P-0025

Investigation into the high prevalence of HCV infection in a rural village in southwest China

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Purpose: To clarify the changing pattern of hepatitis C virus prevalence in China, with the aim of developing appropriate and effective strategies for the prevention and treatment of this significant emerging disease.

Methods: The residents of Village M, located in southwest China, voluntarily participated in this study. Blood samples were obtained and anti-HCV titers were tested to determine the HCV status of the participants. For those who were anti-HCV positive, HCV RNA levels and genotypes were subsequently tested. HBV-related factors and anti-HIV titers were also tested. In addition, the methodology historically used to sterilize needles in the medical center used by the villagers was recreated to test the transmission feasibility of this procedure.

Results: Anti-HCV antibody testing showed that 253 (50.3 %) of the 503 participants were anti-HCV positive, and among which majority (179/253) of anti-HCV positive participants were also HCV RNA positive. Genotyping based on NS5B sequences showed that the predominant subtype of HCV was 3b (96.2 %), followed by 6a (1.9 %) and 1b (1.9 %), respectively. The recreated medical procedure indicated that the transmission route might through inadequately sterilized needles. The HBV infection rate among participants was 34.0 % and the HBV/HCV co-infection rate was 17.5 %, but none of the participants were anti-HIV positive.

Conclusions: HCV infection was highly prevalent in a village in southwest China. A novel transmission or prevalence pattern of HCV infection was identified that might be inadequately sterilized needles. High prevalence of co-infection with HBV among the anti-HCV positive individuals reflects poor medical service in this area.

P-0026

HIV and HCV Cooperatively Promote Hepatic Fibrogenesis through Epimorphin mediated via FAK-ERK

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Background: HIV/HCV coinfection leads to accelerated hepatic fibrosis progression, with higher rates of cirrhosis than does HCV mono-infection. However, the profibrogenic role of HIV on hepatocytes and hepatic stellate cells (HSC) has not been fully clarified.

Methods: LX-2 cells, a human HSC line, were challenged with inactivated HIV and JFH-1 HCV supernatant respectively. We used Cell cycle and Matrigel assays to investigate the effect of HIV/HCV coinfection on proliferation and invasion. The expression of Epimorphin (EPM), fibrogenic markers and downstream intracellular signaling pathways were assessed by qRT-PCR and Western blot. We also performed RNAi and specific pathway inhibitors to evaluate the signaling pathways.

Results: HIV/HCV coinfection promoted hepatic stellate cells proliferation and migration in an EPM-dependent way in vitro. Meanwhile, α -SMA, Collagen, TGF- β and EPM were increased in mRNA and protein level. The decreases of matrix metalloproteinase-3 (MMP-3) and increase tissue inhibitors of MMP 1 (TIMP-1) expression were EPM-dependent. Furthermore, EPM-mediated regulation of MMP-3 and TIMP-1 was through the activation of focal adhesion kinase (FAK)/extracellular signal-regulated kinase (ERK) axis.

Conclusions: Our data provide further evidence that HIV and HCV regulate hepatic fibrosis progression through the generation of EPM in many aspects. HIV and HCV can individually and jointly up-regulate EPM, EPM induces HSCs proliferation and invasion. Furthermore, EPM reduce the potentially antifibrogenic expression of MMP3, as well as induce the profibrogenic genes TIMP1 in an FAK-ERK dependent signal pathway. These findings will support EPM as an important biomarker in activated HSCs for hepatic fibrosis of HIV/HCV infection.

P-0027

Identification of inter-genotype recombinant in Indonesian chronic HCV patient

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Genetic recombination is an HCV RNA feature that plays significant role in their evolution. Few natural inter-genotypic recombinant HCV strains have been characterized. Interestingly, recombination break points were predominantly found within NS2 or around the NS2/NS3 junction with genotype 2 as parental genotype. Our previous study identified inter-genotype recombinant HCV in three out of 150 patients (2 %) with putative genotypes 3 k/2a, 1a/2a and 1b/2a based on the core and NS5B regions. The aim of this study is to fully sequence the putative recombinant strain of HCV 1b/2a. HCV RNA was extracted and genotyped based on Core and NS5B region. Eight separate fragments were synthesized to cDNA followed by nested PCR. The obtained DNA were cloned, sequenced, and analyzed. Further analysis showed that the sample was infected with HCV variant of genotype 1b and putative recombinant 1b/2a. cDNA containing putative genotype 1b/2a was sequenced into six overlapping fragments. The first three fragments which cover the Core-NS2 regions correspond to genotype 1b, while the last three fragments containing NS3-NS5B regions correspond to genotype 2a. In conclusion, this HCV recombinant was the first inter-genotypic recombinant found in Indonesia with a unique feature as it has genotype 1 as parental genotype and recombination breakpoints within NS2-NS3 junction. Recombination between genotype may present a serious predicament as it may effect treatment response and vaccine development.

P-0028

Impact of chronic HCV G1b infection on TG concentration in serum lipoprotein fractions

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Background: Alteration of lipid metabolism in chronic hepatitis C virus (HCV) infection has been investigated. Although reduced low-density lipoprotein (LDL) cholesterol and total cholesterol levels are characteristic features of dyslipidemia in HCV infection, abnormality in serum triglyceride (TG) has not been fully investigated.

Methods: We attempted to clarify the impact of HCV genotype 1b (G1b) infection and advanced liver fibrosis with HCV infection on serum TG profiles, TG concentrations in lipoprotein fractions were examined in fasting sera from 190 subjects with active ($n = 97$) or cleared ($n = 93$) HCV infection by high-performance liquid chromatography. Serum lipoproteins were fractionated into four classes according to the particle size: chylomicron, very low-density lipoprotein (VLDL), LDL, and high-density lipoprotein (HDL). Then, the significance of HCV G1b infection and advanced fibrosis on TG levels in each lipoprotein fraction was determined using multiple regression models.

Results: We found that active HCV G1b infection was positively associated with high HDL-TG levels ($P < 0.001$) and low VLDL-TG levels ($P = 0.01$), independent of other factors included in the regression model. In VLDL sub-fractions, active HCV infection was only found to be associated with low level of large VLDL-TG ($P = 0.01$). Similarly, advanced fibrosis in chronic HCV G1b infection was significantly associated with high levels of LDL-TG ($P = 0.02$) and small VLDL-TG ($P = 0.01$), and marginally associated with high level of HDL-TG ($P = 0.05$), independent of other clinical factors.

Conclusion: These findings indicate that active HCV G1b infection and advanced fibrosis are closely associated with abnormal serum TG profiles.

P-0029

Increased cancer rates in CHC patients: an analysis of the Cancer Registry in a large U.S. HMO

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Background: Hepatitis C virus (HCV) is an oncogenic virus, and an increased risk of malignancy in HCV has previously been reported. Cancer types associated with HCV include non-Hodgkin's lymphoma, renal and prostate cancers; as well as liver cancer. The aim of this study was to describe cancer rates in our cohort of HCV patients compared to cancer rates in the non-HCV population.

Methods: This is a retrospective study at Kaiser Permanente Southern California (KPSC), a large health maintenance organization with 3.5–4 million members. The KPSC cancer registry is an accredited program maintaining a complete profile of all cancer diagnoses for all KP members. In this study, we compared cancer incidence between HCV and non-HCV patients in patients ≥ 18 years of age with or without HCV during 2008–2012.

Results: From 2008 to 2012, 145,210 patient years were included in the HCV cohort versus 13,948,826 for the non-HCV cohort. Mean age at cancer diagnosis in the HCV cohort was 61.8 years (63.5 years for non-HCV cohort). In the HCV cohort there were 2213 cancer diagnoses (1524/100,000) during the 5 year period and 1654 cancer diagnoses when liver cancer was excluded (1139/100,000). In the non-HCV cohort there were 84,419 cancer diagnoses (605/100,000) during the same 5 year period and 83,795 (601/100,000) when liver cancer was excluded.

Conclusions: In our cohort of Hepatitis C infected patients, cancer rates were significantly increased compared to the non-HCV cohort. This suggests that another extrahepatic manifestation of HCV may be an increased risk of cancer.

Table 1. Rate ratios between the HCV and non HCV cohorts for the total number of cancer cases including and excluding liver cancer.

Cancer diagnosis 2008-2012	HCV members N=145210 Rate	Non HCV members N=13948826 Rate	Rate Ratio HCV vs Non HCV	p-value
Head& neck	47.5	16.7	2.85 (2.24,3.62)	<0.0001
Esophagus	10.3	4.4	2.34 (1.40,3.91)	0.0011
Stomach	28.2	9.1	3.10 (2.27,4.23)	<0.0001
Colon/rectum	106.7	55.2	1.93 (1.65,2.27)	<0.0001
Liver	385.0	4.5	86.05 (76.77,96.46)	<0.0001
Pancreas	34.4	12.6	2.74 (2.07,3.63)	<0.0001
Lung/bronchus	111.6	46.8	2.38 (2.04,2.79)	<0.0001
Prostate	174.9	88.4	1.98 (1.75,2.24)	<0.0001
Renal	66.8	20.4	3.27 (2.67,4.00)	<0.0001
Non-Hodgkin's lymphoma	80.6	22.2	3.63 (3.02,4.37)	<0.0001
Myeloma	22.7	7.8	2.93 (2.07,4.14)	<0.0001
All sites, incl liver cancer	1524	605	2.52 (2.41,2.63)	<0.0001
All sites, excl liver cancer	1139	601	1.90 (1.81,1.99)	<0.0001

P-0030

Parvovirus 4 infection in Iranian HCV infected patients and healthy individuals**Seyed Reza Mohebbi¹, Hosna Rastegar^{1,2}, Seyed Masoud Hosseini², Pedram Azimzadeh¹, Shabnam Kazemian¹, Maryam Karkhane³, Mohammad Reza Zali¹**

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Background: Parvovirus 4 (PARV4) is a newly described small DNA virus. Due to the importance of HCV on public health, further studies, including viral coinfections, would be necessary. Thus, in this study, we investigated the prevalence of PARV4 virus among patients with chronic HCV infection compared with healthy controls and related risk factors among these groups.

Methods: A total of 206 patients, including 103 chronic HCV infected patients and 103 healthy controls were studied. Samples were screened for PARV4 DNA using nested PCR (nested primers for ORF2 and ORF1) and also real time PCR (primers and probe for ORF1). Both groups were assessed for the presence of the virus.

Results: Of 103 patients with a mean age of 43.73 ± 13.75 years with hepatitis C, 81 patients (78.6 %) men and 22 (21.4 %) were female. The 103 control subjects, mean age 42.13 ± 12.71 years, which of these, 43 (41.7 %) men and 60 (58.3 %) were female. AST enzyme levels with a mean of 40.45 ± 34.84 and 18.58 ± 5.9 in patients and healthy group respectively, no significant difference was found ($P = 0.063$). Finally, after screening all DNA samples from patients and controls, we discovered that none of these people are infected with the PARV4 virus.

Conclusions: This study is the first to investigate the occurrence of PARV4 among healthy population and HCV patients in Iran. The results show that, the virus is not important in Iranian population, even in patients with blood born infections such as HCV and further studies in other areas and various risk groups is required.

P-0031

Intrahepatic HCV RNA and genotype 1 independently associate with hepatic reticulon 3 expression**Chih-Lang Lin^{1,2,3}, Chau-Ting Yeh^{2,3}, Rong-Nan Chien^{1,3}, Kung-Hao Liang², Ya-Hui Huang², Po-Yuan Ke³**

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Background: The reticulon protein 3 (RTN3) is residing predominantly in endoplasmic reticulum. It could interact with hepatitis C virus (HCV) NS5A and NS4B proteins respectively, but with a conflicting regulatory role in HCV replication. To gain insight into this issue, clinical samples obtained from patients with HCV-related

hepatocellular carcinoma (HCC) were used to investigate the correlation between HCV RNA level and RTN3 expression.

Methods: From July 2002 to August 2007, 115 HCV-associated HCC patients receiving surgical resection were enrolled. All of them were negative for hepatitis B virus (HBV) surface antigen. The hepatic HCV RNA and RTN3 protein levels were assayed using the non-cancerous parts of liver tissues.

Results: Of the 115 enrolled patients, occult HBV infection (positive for tissue HBV DNA) was detected in 16 (11.5 %) patients. Univariate followed by multivariate analysis revealed that intrahepatic RTN3 levels were independently associated with higher HCV viral load (odds ratio [OR] = 1.053; 95 % confidence interval [CI] = 1.009–1.099; $P = 0.018$) and HCV genotype 1 (OR = 2.846; 95 % CI = 1.202–6.734; $P = 0.017$). Multivariate Cox analysis revealed that HCV genotype 1 ($P = 0.001$), tumor size >4.4 cm ($P = 0.018$), albumin <3.9 g/dL ($P = 0.028$), and aspartate transaminase >51 U/L ($P = 0.001$) were associated with a shorter recurrence-free survival. Only alpha-fetoprotein >38 ng/ml ($P = 0.018$) was associated with a shorter overall survival.

Conclusions: Higher intrahepatic RTN3 levels were independently correlated with higher intrahepatic HCV RNA levels and genotype 1 HCV. However, it could not serve as a predicative role for postoperative prognosis.

P-0032

There an increase in the prevalence of hepatitis C virus subtype 3a in Turkey**Metin Basaranoglu¹, Serife Yuksekkaya², Fatma Kalem³, Aysegül Opus², Mahmut Kurtoglu²**

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Background/aims: Hepatitis C virus (HCV) causes liver infection and lead to cirrhosis and liver cancer in some. For therapeutic management; HCV genotypes must be known. In this study, we aimed to evaluate the distribution of HCV genotypes by time.

Materials and methods: In this study the distribution of Hepatitis C virus genotypes were determined of 314 patients with chronic HCV infection who had admitted to Central Microbiology laboratory of Konya Training and Research Hospital in Turkey between January 2011 and December 2014. HCV genotypes were determined by using a commercial LiPA kit based on the reverse hybridization of amplification products of viral 5'-UTR region.

Results: The study population consisted of 314 patients with chronic HCV infection. Among them, 153 (48.7 %) were males and them 161 (51.3 %) were females. In 244 (77.7 %) patients Genotype 1 and in 224 patients genotype 1b was determined. Genotype 1b was the most common genotype. The second most common genotype was 3a. Genotype 3a is firstly seen in 2013 and there is an increase in 2014. Distribution of Hepatitis C virus genotypes of 314 patients according to years was shown in Tables 1 and 2 and figure 1.

Conclusion: HCV infection is an important public health problem. Both, planning the treatment and monitoring the distribution of HCV genotypes are necessary. According to genotype; treatment, treatment duration and response to treatment are changeable. So, to be know HCV genotype provides important epidemiological and therapeutic information.

P-0033

Investigation of the effect of PON1 and IL28B gene polymorphism on HCV infection progress

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This study, effect of IL28B and PON1 gene polymorphisms on HCV infection process were investigated. The polymorphisms rs12979860 and rs8099917 which are localized on IL28B gene region and also rs854560 (55th region) and rs662 (192nd region) on PON1 enzyme were detected. Thirty six spontaneously meliorated and 48 chronic patients were included to our study. The research was planned as a attitude determining study. Deoxyribonucleic acid isolation was carried out by 'spin column' method. About 4 gene polymorphisms in IL28B gene and PON1 polymorphisms were determined by genotyping kit. PON1 polymorphism analysis results: spontaneous clearance, 55th position MM genotype 12 (33.33 %), LM genotype 16 (44.44 %), LL genotype 8 (22.22 %) ($p < 0.05$). 192nd position QQ genotype 20 (55.55 %), QR genotype 11 (30.55 %) and RR genotype be determined 5 (13.89 %). ($p > 0.05$). rs12979860 polymorphism of IL28B gene region were determined in 19 spontaneous clearance (52.78 %) with CC genotype at rs8099917 polymorphism in the 23 (63.89 %) were higher with the TT genotype. M/L55 and combined polymorphism at (LL/QR, MM/RR, MM/QR) relationship between chronic HCV cases and spontaneously meliorated group were found statistically significant ($p < 0.05$). Q/R192 polymorphism were not found significant differences ($p > 0.05$). As a result, M/L55 polymorphism of MM genotype or MM/RR and MM/QR are combined genotypes for PON1 and IL28B rs12979860 polymorphism in patients with HCV those have CC genotype; we found that spontaneous clearance developments increase the likelihood. These polymorphisms will prevent unnecessary treatments by facilitating the uptake of treatment initiation decision on the patients with HCV infection.

P-0034

Length of TA repeat near IL28B was associated with HCV spontaneous clearance in Egyptian population

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Background: We previously reported that the length of the TA dinucleotide repeat in the promoter region of IL28B has a wide variation. Although the distributions of TA repeat greatly differed between Japanese and African-American, a long TA repeat was

associated with spontaneous HCV clearance both in two populations. In this study, we determined the TA repeat of Egyptian people to validate the relation to spontaneous HCV clearance in a different race. **Methods:** Total 160 Egyptian genomic samples were enrolled (80 with HCV spontaneous clearance and 80 with chronic HCV infection). The length of TA repeat was determined by 3130xl sequencer and GeneMapper software.

Results: The distribution of TA repeat ranged from 6 to 18 as well as African-Americans. The most frequent repeat was 12 (39.1 %) similarly to Japanese (82.8 %) and African-Americans (28.9 %) although the percentages differed greatly. By the meaningful cut-off number of ten for the previous two populations, we divided Egyptians into two groups. Then, the persons with TA repeat under 11 showed a lower probability of spontaneous clearance, however the difference did not reach the significance (41.9 vs. 53.4 %, $P = 0.13$). On the other hand, dividing Egyptians by thirteen, the persons with TA repeat over 13 had a significant higher probability of spontaneous clearance (55.5 vs. 39.2 %, $P = 0.04$).

Conclusions: A long TA repeat in the promoter region of IL28B was associated to HCV spontaneous clearance in Egyptian population as well as other population. However, the significant cut-off numbers of TA repeat were different by races and ethics.

P-0035

Metformin activates type I interferon signaling against HCV via activation of AMPK

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Background: Activation of the type I interferon (IFN) signaling pathway is essential for the eradication of hepatitis C virus (HCV). Metformin can activate adenosine monophosphate-activated protein kinase (AMPK) to reduce IR. Cross talks between AMPK and IFN signaling remain unclear.

Materials and methods: The full-length HCV replicon OR6 cells and the infectious HCV clones JFH1 were used to assess the anti-HCV effect of the insulin sensitizers, metformin and pioglitazone. The key steps of the IFN signaling pathway activated by metformin were characterized with immunoblotting analysis. Finally, influence of AMPK inhibitor on IFN signaling following metformin treatment was determined.

Results: Immunofluorescence staining and the immunoblotting of HCV viral protein and real-time PCR of HCV viral RNA, demonstrated that metformin, but not pioglitazone, inhibited HCV replication in OR-6 and JFH-1 infected Huh 7.5.1 cells. Metformin activated the phosphorylation of STAT-1 and STAT-2, leading to the expression of IFN-stimulated genes, ISG15, MxA and PKR. Metformin enhanced the phosphorylation of AMPK and metformin activated IFN signaling was down-regulated by AMPK inhibitor. After treatment of AMPK inhibitor, the level of HCV core protein decreased by metformin can be rescued.

Conclusions: metformin activates type I interferon signaling and inhibits the replication of HCV via activation of AMPK.

P-0036

New-estimation of prevalence of HCV infection in Mongolia**Dashtseren Bekhbald^{1,2}, Oidovsambu Odgerel^{2,3}, Dashdorj Naranjargal³, Dagvadorj Tungalag¹, Bold Bayarmagnai^{1,2}, Yagaanbuyant Dahgwahdorj^{1,2}**¹Department of Immunology, Mongolian National University of Medical Sciences, Ulaanbaatar, Mongolia; ²Liver Center, Ulaanbaatar, Mongolia; ³Mongolian National University, Ulaanbaatar, Mongolia**Introduction:** Prevalence of HCV infection in Mongolia as hyper-endemic well known. In almost publication about it cited the data collected more 10 years ago, published in Hepatology International in 2006. But, many measurement against to this infection by government and non-government organization in this time. Aim of study: re-estimate prevalence of HCV infection in Mongolia.**Methods and subjects:** This population-based study includes total of 1158 apparently healthy people (above 20 years old), which randomly selected. Screening for anti-HCV were performed by ELISA and HCV-RNA were measured by Abbott m2000sp/m2000rt system in Liver Center.**Results:** Total of 1158 subjects were enrolled including 599 (43.1 %) men and 659 (56.9 %) female. The overall prevalence of anti-HCV among study subjects was 11.1 % (128/1158). Higher percentage of female subjects (12.6 % of female) were tested positive in comparison to the 9 % of male subjects. Also 11.1 % (128 individuals) were tested as anti-HCV positive and 84 % (103 individuals) were HCV-RNA positive from them. Results of multivariate regression analysis for potential risk factors show that history of blood transfusion 1.5 (OR = 1.563 95 % C.I. 1.060–2.305 p = 0.024), acupuncture 1.3 times (OR = 1.303 95 % C.I. 1.110–1.531 p = 0.001), letting blood treatment 3.5 times (OR = 1.878 95 % C.I. 1.427–2.471 p = 0.0001) and surgical procedure (OR = 3.513 95 % C.I. 2.163–5.704 p = 0.0001) were associated with significant risk for transmission of HCV.**Conclusion:** It is estimated that currently in Mongolia approximately 114000 adult people infected with HCV. The risk factor analysis show that the nosocomial infection is the leading risk factor of HCV infection in Mongolia.

P-0037

Clinical characteristics of chronic hepatitis C in the elderly patients**Sungbum Cho, Chunghwan Jun, Eunae Cho, Hyungmin Rew, Kyunglee Kim, Sungkyu Choi**

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Background and aims: the present study aimed to elucidate clinical characteristics and feasibility of antiviral treatment of the elderly patients with chronic hepatitis C.**Patients and methods:** A total of 1388 patients (Age >65, n = 668, 48.1 %) with chronic hepatitis C from January 2004 to December 2014 were reviewed.**Result:** The distribution of genotypes was as follows: 1.8 % of genotype 1a; 39.3 % of 1b; 3.1. of 1c; 0.6 % of genotype single 1; 2.4 % of 2a; 38.1 % of 2a/2c; 11.3 % of genotype single 2; 0 % of genotype 3; 1.2 % of genotype 4; 0 % of genotype 6; and 2.4 % of non-applicable. Cirrhosis, decompensation, and hepatocellular carcinoma are more frequently in the elderly compared to the non-elderly

patients (26.5 vs. 17.2 % / 14.8 vs.6.0 %/6.8 vs. 3.4 %, p < 0.001). Fifty (7.5 %) elderly patients received antiviral treatment with pegylated interferon and ribavirin compared to 257 (35.7 %) non-elderly patients (p < 0.001). The proportion of treatment discontinuation was 18.0 % (9/50) in the elderly vs. 19.1 % (49/2567) in the non-elderly (p = 1.000). Cytopenia occurred in 76 % (38/50) in the elderly vs. 67.1 % (171/257) in the non-elderly (p = 0.476). The sustained virologic response rate of treated patients was 52.0 % (26/50) in the elderly vs. 64.6 % (166/257) in the non-elderly (p = 0.294).

Conclusion: Treatment attempt was lower in the elderly patients compared to the non-elderly patients. However, treatment discontinuation rate, cytopenia, and the SVR rate were similar between the elderly and the non-elderly patients. This study may facilitate treatment options in the elderly patients with chronic hepatitis C.

P-0038

PNPLA3 genetic variants determine HCV-related hepatic steatosis in Asian non-obese patients**Chung-Feng Huang^{1,2}, Chia-Yen Dai¹, Jee-Fu Huang¹, Wan-Long Chuang¹, Ming-Lung Yu¹**¹Hepatobiliary Division, Department of Internal Medicine, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan; ²Department of Occupational Medicine, Kaohsiung Municipal Ta-Tung Hospital, Kaohsiung Medical University Hospital, Kaohsiung Medical University, Kaohsiung, TaiwanThe influence of patatin-like phospholipase domain-containing 3 (PNPLA3) genetic variants in the development of hepatitis C virus (HCV)-related liver steatosis in Asian ethnicities with different demographic characteristics remains elusive. A total of 1018 biopsy-proven chronic hepatitis C patients were enrolled for evaluation. The proportions of PNPLA3 rs738409 GG genotype carriage were 7.8 % (44/563), 15.8 % (58/367) and 19.3 % (17/88) in patients with no (liver fat content less than 5 %), mild (5–33 %) and moderate/severe (more than 66 %) hepatic steatosis, respectively (trend P < 0.001). Stepwise logistic regression analysis revealed that the strongest factor independently associated with steatosis was the carriage of the PNPLA3 rs738409 GG genotype (odds ratio [OR]/95 % confidence intervals [CI]: 2.34/1.557–3.515, P < 0.001). Among the patients with BMI <24 kg/m², carriage of the rs738409 GG genotype was the only factor associated with hepatic steatosis (OR/CI: 3.44/1.824–6.500, P < 0.001). PNPLA3 genetic variants had minimal effects on hepatic steatosis among overweight or obese patients. Compared to patients with BMI <24 kg/m²/non-GG genotype, those with BMI >24 kg/m²/GG genotype were more likely to have hepatic steatosis (OR/CI: 3.87/2.292–6.524, P < 0.001). In conclusions, both PNPLA3 genetic variants and BMI played important roles in HCV-related hepatic steatosis among Asian patients. However, the genetic effect was mainly restricted to non-obese patients.

P-0039

Genotype distribution of hepatitis C virus in HIV infected patients in Bydgoszcz, Poland**Anita Olczak¹, Malgorzata Tyczyno², Edyta Grabczewska¹**¹Departament Infectious Diseases and Hepatology Collegium Medicum UMK, Bydgoszcz, Poland; ²Wojewodzkiej Szpital

Obserwacyjno-Zakazny w Bydgoszcz, Collegium Medicum, Nicolaus Copernicus University, Bydgoszcz, Poland

Introduction: Due to shared routes of transmission, co-infection with human immunodeficiency virus type 1 and hepatitis C virus is common. It results in an accelerated rate of liver disease-related morbidity and mortality. Management of HIV-HCV coinfection is complex due to the nature of antiretroviral and HCV therapy, and drug-drug interactions between new HCV direct-acting antiviral therapies and antiretroviral regimens and hepatotoxicity of antiretroviral drugs.

Objectives: The aim of the study was to determine the distribution of HCV genotypes among patients infected with human immunodeficiency virus-1.

Methods: HCV genotypes were identified in HCV RNA positive samples obtained from HIV infected patients who attended to Department of Infectious Diseases in Bydgoszcz in years 2002-2014. HCV genotype was performed using LINEAR ARREY assay (Roche, Mannheim, Germany) after isolation and amplification of the material with COBAS AMPLICOR v 2.0 (Roche, Mannheim, Germany).

Results: 152 anti-HCV positive serum samples were selected for subsequent HCV RNA detection and genotyping. HCV RNA could be detected in 139 (91.5 %) of 152 samples. Genotype 3 was predominant (38 %) followed by genotype 1 (34.6 %) and genotype 4 (27 %). The mixed infection was detected in 2 cases (1.4 %).

Conclusion: The current study shows predominance of genotype 3 among HIV/HCV, but it should be stressed that genotype 4 was the third most prevalent genotype in this population. Ongoing monitoring of the epidemiology of HCV infection in HIV infected population is a crucial for treatment strategy and preventing future infections in Poland. Treatment for hepatitis C is strongly encouraged early in all coinfecting patients.

P-0040

Performance evaluation of the HISCL® HCV serotyping assay in Chinese chronic hepatitis C patients

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Objective: HCV genotype is a crucial baseline factor to guide the treatment regimen and predict the outcome for chronic hepatitis C patients. An automated assay, the HISCL® HCV serotyping assay, was developed for detecting genotype 1 and 2. The performance was evaluated.

Methods: There were 245 consecutive routine clinical samples genotyped using the Versant HCV Genotype 2.0 Assay. They were subsequently serotyped. The consistent results yielded by both assays were considered as the confirmed results, whereas the inconsistent results were confirmed by the phylogenetic analysis.

Results: There were 114 genotype-1 samples, 53 genotype-2 samples, 39 genotype-3 samples and 39 genotype-6 samples. For the 167 genotype-1 or -2 samples, 24 samples failed to be serotyped using the assay (i.e., failed to be detected or generated indeterminate result); ten or 8.77 % genotype-1 samples and 14 or 26.42 % genotype-2 samples failed to be serotyped. For the remaining 143 samples, the consistency was 98.60 % (141/143) between the assays; two or 1.75 % genotype-1 samples was misclassified as genotype-2 samples, and no genotype-2 samples were misclassified using the serotyping assay. For the 78 genotype-3 and -6 samples, the assay misclassified 10 or 25.64 % genotype-3 samples as genotype-1 samples, and misclassified 26 or 66.67 % genotype-6 samples as genotype-1 or -2 samples.

Conclusions: The assay accurately determines HCV genotypes 1 and 2. But it is likely to misclassify some genotype-3 samples as genotype-1 samples, and had low specificity for genotype 6. Therefore, it is not recommended to be used where the genotype 3 or 6 is prevalent.

P-0041

Prevalence and clinical correlates of autoantibodies in Chinese patients with chronic hepatitis C

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Background: Autoantibodies are frequently found in the sera of patients with Hepatitis C Virus (HCV) infection. However, no conclusive answers have been produced concerning the clinical relevance of these antibodies. To determine whether a relationship might exist between the presence of autoantibodies and the severity of liver disease in HCV.

Methods: A total of 1009 consecutive treatment-naïve Han ethnic adults with chronic HCV infection enrolled at 28 hospitals across China were studied. The presence of anti-nuclear antibodies (ANA), anti-smooth muscle antibodies (SMA) and anti-mitochondrial antibody (AMA) were screened by indirect immunofluorescence. Serum HCV RNA was detected using real-time RT-PCR. HCV genotype was measured with The Versant HCV Genotype 2.0 Assay. Routine clinical biochemistry were assayed by an automated kinetic method.

Results: ANA, SMA, and AMA occurred in 50.9, 3.9, and 2.0 % of cases respectively. In women, a higher percentage of patients were tested for autoantibodies as compared with men ($p < 0.001$). No specific association between autoantibodies and a specific HCV genotype was found. Patients positive for autoantibodies were significantly older ($p < 0.001$) and had higher mean (SD) TP levels ($p < 0.001$) and lower ALB levels ($p < 0.001$) and lower mean (SD) HCV RNA levels ($p < 0.001$) than those without autoantibodies. Autoantibodies reactivity was shown to be independently associated with abnormal levels of AST ($p < 0.05$). AST alteration was associated also with ANA reactivity ($p < 0.05$).

Conclusions: The positive rate of autoantibodies is correlated with age, sex, virus reproduction. Autoantibodies were associated with the most severe forms of chronic HCV infections.

P-0042

Prevalence of HCV and HBV, and characteristics of HCV genomes among general population in Cambodia

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Background: Hepatitis B virus (HBV) and C virus (HCV) persistent infection are the major cause of developing cirrhosis and

hepatocellular carcinoma. Liver cancer is a major cause of cancers in Cambodia. Since 2010 we have conducted the survey to investigate hepatitis viral infections among general population in Cambodia. This study aimed to clarify the characteristics of HCV genomes among general population in Cambodia by sequencing full-length genomes.

Methods: From 2010 to 2014 epidemiological survey consisting of blood sampling and questionnaire was conducted at four areas in Siem Reap Province. HBsAg, anti-HBc, anti-HBs, anti-HCV and quantity of HBV DNA and HCV RNA were tested. Genotypes of HCV were determined by phylogenetic analysis and the full-length genomes of HCV were determined by direct sequencing. This study was approved by the ethical committees of Hiroshima University, Japan and Ministry of Health, Cambodia.

Results: 868 participants consisted of 508 female and 360 male (age range 7–90). Prevalence of HBsAg was 4.7 %, and HBV genotypes were genotype C (87.9 %) and genotype B (3.0 %). Prevalence of anti-HBc, anti-HBs were 28.4 %, 24.8 % respectively. Prevalence of anti-HCV, HCV RNA were 3.9, 1.3 %. HCV was identified genotype 1b (36.4 %) and genotype 6 (63.6 %), the detail of genotype 6 was 6e (28.6 %), 6q (14.3 %), 6r (42.9 %), 6 s (14.3 %).

Conclusion: In northwest Cambodia the prevalence of HBsAg and HCV RNA were 4.7 and 1.3 %, respectively. We have determined full genome sequences of HCV RNA positive participants whose genotype 1b or 6e and will show the characteristics of HCV genomes among the general population in Cambodia.

P-0043

Prevalence of hepatitis-C virus infection within Community Health Centers in Tangerang, Indonesia

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Aim: The epidemiological study of Hepatitis-C virus infection in Tangerang Regency, Indonesia, with a population of approximately 2.8 Millions is still limited. The aim of this study is to study the prevalence of Anti Hepatitis C Virus (HCV) among patients who came to Community Health Center in the urban and rural areas of Tangerang Regency.

Material and methods: A total of six Community Health Center in urban and rural areas were selected. The sample recruitment were 15–60 years old male and female, with no history of alcohol use and of jaundice. Patients' plasma was screened for anti-HCV, HCV RNA and HCV Genotype. Anti-Hepatitis C Virus antibody was detected using rapid anti-Hepatitis C Virus test strip. Hepatitis C Virus RNA level was determined with real time PCR whilst Hepatitis C Virus genotyping was done using direct sequencing.

Results: Total of 884 patients were enrolled in this study, 393 from the urban areas and 491 from the rural areas. Screening test for detection of Anti HCV, HCV RNA and HCV Genotype from the serum showed only two patients from urban areas positive to anti HCV with genotype 1a and 1b. Their viral load were 2.1×10^7 IU/mL and 1.3×10^7 IU/mL; and ALT were 47 and 20 U/L respectively.

Conclusion: The prevalence of Anti HCV-positive showed only 0.23 % in urban and 0 % in rural is relatively low in Tangerang Regency.

Key words: Anti-HCV, Tangerang regency, Community health center

P-0044

Prevalence of TA repeat, IL28B, IFNL4 and correlation with treatment outcome of HCV infection

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Background: The interleukin-28B (*IL28B*) gene polymorphism is a strong baseline predictor of SVR in hepatitis C virus (HCV) treatment. The length of thymine-adenine dinucleotide repeats, or (TA)_n, in the regulatory region of *IL28B* can affect interferon transcription. In order to determine predictive values in HCV infection, we explored the correlation among factors including (TA)_n genotypes, clinical features, interferon- λ -3 (IFNL3) and interferon- λ -4 (IFNL4) polymorphisms, and HCV treatment outcome.

Methods: Sera from 492 patients with chronic HCV infection, 101 individuals with spontaneous HCV clearance and 123 healthy blood donors (control group) were analyzed. Genotyping of the (TA)_n was performed by direct sequencing. The rs12979860 (IFNL3) was identified using nested PCR and sequencing, while ss469415590 (IFNL4) was identified by real-time PCR.

Results: The distribution of (TA)_n was similar between individuals with spontaneous HCV clearance and chronic HCV infection, but differed significantly from healthy controls. Individuals with both (TA)_n alleles ≥ 12 had significantly higher SVR rate compared to individuals with at least one (TA)_n < 12 allele. This strong correlation was seen for patients infected with HCV-1, HCV-3, HCV-6. The (TA)_n genotypes were not associated with HCV viral load, ALT levels and liver stiffness, but were correlated with platelet counts ($p < 0.001$). In contrast, rs12979860 (CC) and ss469415590 (TT/TT) genotypes were associated with higher SVR rate only in patients with HCV-1.

Conclusions: The (TA)_n genotypes were not associated with spontaneous clearance of HCV infection but associated with treatment response in patients infected with HCV-1, HCV-3, HCV-6. In contrast, IFNL3 and IFNL4 polymorphisms were predictive of treatment outcome only for patients infected with HCV-1.

P-0045

Results of long-term monitoring of treatment-naïve genotype 1b chronic hepatitis C patients

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Background and aims: Chronic hepatitis C (CHC) is a slowly progressive disease, the most important complications of which are gradual cirrhosis and HCC. The Peg IFN + Rib combination was until recently the only therapeutic option. The virological response rate in genotype 1b patients is approximately 40 %. This study assessed the long-term monitoring results of treatment-naive genotype 1b patients and compared these with those of treated patients.

Materials-methods: Records of CHC patients monitored in our out-patients clinic were examined, and treatment-naive patients and treated patients identified as attending at least two visiting a year were included in the study. Patients' demographic, clinical, virological, biochemical features, transient elastography (TE) and non-invasive serum biomarkers results were analyzed. Of these patients follow-up period ranged from 6–14 years.

Results: Ninety-eight treatment-naive patients and 128 treated patients had inclusion criteria. Follow-up period of these patients ranged from 6 to 14 years. Patients' demographic, virological and biochemical characteristics, serum biomarkers and (TE) results are shown in the table. Age of patients started on treatment was lower than that of patients monitored without treatment ($p = 0.001$) and HCV RNA ($p = 0.001$) and ALT ($p = 0.032$) levels were higher. There was no difference between the two patient groups in terms of other findings.

Conclusion: Our results show that there is no difference in terms of course of fibrosis between treatment-naive patients with genotype 1b CHC and those receiving peg-IFN + ribavirin. These results support the idea that with new therapeutic options it is not too late to treat these patients.

P-0046

Serum survivin and TIMP-1 as non-invasive markers of HCV induced liver fibrosis

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Background: HCV induced liver fibrosis is assessed by liver biopsy which is an invasive procedure. Tissue inhibitor metalloproteinase-1 (TIMP-1) and survivin play a role in the process of fibrosis through affection of extracellular matrix and hepatic stellate cell apoptosis.

Aim of the work: To estimate the value of TIMP-1 and survivin as fibrosis markers and their relation to fibrosis stages in chronic HCV patients.

Patients and methods: This prospective case control study included 100 patients with chronic hepatitis C subjected to thorough clinical evaluation and ultrasound guided liver biopsy by experienced gastroenterologist. Liver biopsies were examined and classified according to METAVIR score by experienced pathologists. Also this study included 25 healthy volunteers. Serum TIMP-1 and survivin protein levels were measured by western blot and ELISA for the patients and the healthy volunteers.

Results: According to METAVIR score 15, 25, 27, 20, 13 patients had F0, F1, F2, F3 and F4 respectively. Serum TIMP-1 was significantly higher in all fibrosis stages (F1–4) than F0 (57 ± 3.6) and control group (52 ± 4.1) ($p < 0.01$). Also, serum TIMP-1 was significantly increasing with progress of fibrosis from F1 (73 ± 3.4), F2 (91 ± 3.3), F3 (116 ± 3.8) to F4 (132 ± 4.3) ($p < 0.001$). Serum

survival also was significantly higher in all fibrosis stages (F1–4) than F0 (1274 ± 94) and control group (1083 ± 88) ($p < 0.0001$). Also, serum survivin was significantly increasing with progress of fibrosis from F1 (1757 ± 116), F2 (2445 ± 94) to F3 (3120 ± 96) ($p < 0.001$) with no significant difference between F3 and F4 (3320 ± 91) ($p > 0.05$).

Conclusion: TIMP-1 and survivin could be used as non-invasive markers of liver fibrosis.

P-0047

The relation of serum vitamin A level with Chronic Hepatitis C

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In our study we aimed to research the relation of serum vitamin A levels with the liver fibrosis caused by hepatitis C virus and interferon based chronic hepatitis C (CHC) therapy response.

Methods: Study data were obtained from four tertiary centers of Turkey. Consecutively 148 patients who were given interferon therapy were included in the study retrospectively. Serum basal vitamin A levels were also assessed before treatment. 44 healthy volunteer donor bloods were included as control group.

Results: In total, the data of 133 CHC patients were evaluated. The mean baseline serum vitamin A levels with CHC patients were 87.72 ng/mL while mean vitamin A levels were 155.64 ng/mL in healthy control group. The mean baseline serum vitamin A levels of CHC patients compared to control group were detected to be lower at a statistically significantly ($p = 0.0001$). When vitamin A levels of sustained virological response (SVR) positive and SVR negative patients were compared, there was no significant difference. The mean basal serum vitamin A levels according to liver fibrosis (F) levels were detected to be F1; 64.6 ng/mL, F2; 80.6 ng/mL, F3; 91.3 ng/mL, F4; 68.4 ng/mL F5; 116.8 ng/mL F6; 138.4 ng/mL, respectively. When the mean serum vitamin A levels at F1 were compared with F5–6 levels, it was significantly lower ($p = 0.008$).

Conclusion: Serum vitamin A levels of patients with CHC were low and these patients have serum vitamin A deficiency. Liver fibrosis severity at patients with CHC was also associated with serum vitamin A levels and it was higher at patients with severe fibrosis. Serum A vitamin levels wasn't associated with response to CHC treatment.

P-0048

The role of 25-hydroxy vitamin D in hepatitis C virus replication and treatment viral kinetics

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Background: The role of vitamin (vit) D in hepatitis C virus (HCV) infection remains to be elucidated.

Methods: Con1 cells and J6/JFH cells were treated with 1, 5, and 10 μM 25-OH Vit D, respectively. Cell survival was observed via WST1 test and viral loads were observed through Luciferase assay. We recruited retrospectively 3 groups of patients with different treatment responses (Group A: negative both for rapid virological response and sustained virological response [RVR-/SVR-]; Group B: RVR +/SVR-; Group C: RVR +/SVR +) to assess the difference of 25-OH Vit D between groups.

Results: Measured by luciferase assay, the replication ability in 1, 5, and 10 μM 25-OH Vit D treated levels decreased by 69, 80, and 86 %, respectively ($p < 0.0001$) without significant changes on cell viability in WST1 test. The replication ability in 1, 5, and 10 μM 25-OH Vit D treated levels decreased by 12, 55, and 80.5 %, respectively ($p < 0.0001$) in J6/JFH cells. There was a significant incremental increase of 25-OH Vit D between CHC groups, ranging from 4.4 ± 5.6 ng/mL of group A ($n = 44$) patients, 17.2 ± 11.6 ng/mL of group B ($n = 44$) patients, to 32.5 ± 37.5 ng/mL of group C ($n = 44$) patients ($P < 0.001$).

Conclusions: 25-OH Vit D decreases HCV replication in both genotype 1b and 2a replicon cell lines. It also contributes to treatment responses and viral kinetics in anti-viral therapy.

P-0049

Effect of antiviral therapy on liver stiffness in patients of chronic hepatitis C

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Background: Progression of chronic hepatitis C (CHC) is determined by liver fibrosis, usually assessed by liver biopsy (LB), an invasive procedure. Fibroscan is a noninvasive alternative.

Aims: (1) To study effect of antiviral therapy (Pegylated Interferon and Ribavirin) on Liver stiffness (LS) in CHC patients (2) Determine whether baseline LS predicts sustained viral response (SVR).

Methods: We prospectively enrolled 61 patients with CHC in Gastroenterology department D.M.C.H, Ludhiana from Jan 2013–June 2014. LS measurements done at baseline, end of treatment and 6 months after end of therapy.

Results: Of 61 patients enrolled, 48 were males with mean age 42.1 years. Mean fibroscan value at baseline was 9.87 ± 7.62 kPa. In 56 patients with S.V.R., liver stiffness decreased significantly at end of therapy (8.97 ± 6.84 kPa) vs baseline (10.16 ± 7.85 kPa, $p = 0.007$). The decrease was maintained in responders at SVR (8.67 ± 6.84 kPa vs. 10.16 ± 7.85 kPa, $p = 0.04$). In relapsers no significant change in LS at end of treatment (9.71 ± 28.97 kPa, $p = 0.159$) and at SVR (9.06 ± 11.84 kPa, $p = 0.264$) as compared to baseline (9.84 ± 3.05 kPa) observed. Reduction in LS was significant in moderate (Fs 7.1–9.4 kPa) and severe (Fs 9.5–12.4 kPa) fibrosis ($p: 0.009$ and 0.006 respectively) as compared to mild or no fibrosis (Fs < 7.1 kPa, $p = 0.313$) and cirrhosis (Fs > 12 kPa, $p = 0.202$). No significant change in LS with BMI, age, sex and ALT levels observed. No statistically significant difference found between baseline fibroscan values of responders and relapsers (10.16 ± 7.85 vs 9.84 ± 3.05 $p = 0.834$).

Conclusion: 1. Liver stiffness decreased significantly in CHC patients with moderate to severe fibrosis who achieved SVR 2. Baseline fibroscan does not predict SVR.

P-0050

Sequential measurement of liver stiffness in patients with HCV with or without sustained response

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Background and aims: Sequential evaluation of liver fibrosis during clinical course is essential for the management of chronic liver diseases. We retrospectively analysed liver stiffness measured by FibroScan in patients with hepatitis C.

Method: We re-evaluated a previously reported cohort of patients in whom liver stiffness was measured from December 2004 to January 2005 at our hospital. We also added patients who visited our unit from September 2014 to August 2015. Among those patients we enrolled those in whom liver elasticity was measured at least twice during follow-up period. We excluded patients who had already achieved sustained virological response (SVR) at the first measurement of liver stiffness. We assessed the progression or regression of liver stiffness based on the status of SVR and predictive factors for disease progression.

Results: Among 710 patients who met inclusion criteria (M/F: 325/385, mean age: 64.6 years), 154 (21.7 %) achieved SVR during median follow-up period of 6.1 years; 86 (12.1 %) could not achieve SVR instead of anti-viral treatments and 470 (66.2 %) did not undergo anti-viral treatments. Liver stiffness regressed at a rate of 5.3 % per year in the SVR group and progressed at a rate of 2.4 % per year in the non-SVR group. Liver stiffness at study entry and SVR status were correlated significantly with disease progression.

Conclusions: Liver stiffness measurement is a promising tool to monitor disease progression and improvement in patients with chronic hepatitis C.

P-0051

Simeprevir- and telaprevir-based therapies for non-responders with genotype 1b chronic hepatitis C

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Background and aims: The aim of this study was to evaluate the efficacy analysis in the prior or null responders treated with simeprevir and telaprevir-based triple therapies in a prospective, multicenter cohort study.

Methods: This prospective, multicenter study has enrolled 885 genotype 1b patients with chronic hepatitis C who received 12-week triple therapy that included simeprevir ($n = 345$) or telaprevir ($n = 540$) combined with PEG-IFN α 2b and RBV followed by a

12-week dual therapy that included PEG-IFN α 2b and RBV. A total of 155 patients with prior or null response were analyzed, 48 and 107 receiving simeprevir and telaprevir-based therapies, respectively. Serum HCV RNA level was measured by COBAS TaqMan HCV test. **Results:** The overall rate of SVR12 W was only 49.7 % (simeprevir-based therapy 47.9 % and telaprevir-based therapy 50.5 %, respectively). The SVR12 W rate for prior responders (62.7 %) was significantly higher than for null responders (34.7 %) ($P < 0.001$). For prior responders, multivariate analysis extracted interleukin 28B (IL28B) (rs8099917) TT and no or mild advanced fibrosis (F0–2) as independent factors associated with SVR12 W. In fact, the SVR12 W rates for IL28B TT and no or mild advanced fibrosis were extremely high (81.5 and 80 %, respectively). For null responders, multivariate analysis extracted only no or mild advanced fibrosis as independent factors associated with SVR12 W. The SVR12 W rate for no or mild advanced fibrosis was significantly higher (72.2 %) than advanced fibrosis (F3–4) (17.9 %) ($P < 0.001$). **Conclusions:** The response of such patients is associated with the fibrosis stage. IL28B is predictive for achieving an SVR for prior responders.

P-0052

Role of IL-28B genetic variants in HCV related liver disease severity

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Background: The role of host interleukin 28B (IL-28B) in liver disease severity in patients of chronic hepatitis C (CHC) is conflicting. Its impact on Asian patients with different viral genotypes remains elusive.

Aims: To elucidate the effect of IL-28B genetic variants in a large Asian cohort with different viral genotype.

Methods: 1288 biopsy proven CHC patients were enrolled for testing the association of liver fibrosis and IL-28B rs8099917 genotype.

Results: HCV genotype 1 (HCV-1) infection accounted for 59.4 % of the patients and the remaining 518 patients (40.6 %) were with HCV non-1 infection (the majority were with HCV-2 infection). Of the 1084 patients with IL-28 genotype available, nine hundred and twenty eight (85.6 %) patients were with TT genotype. Univariate analysis revealed that patients with advanced liver fibrosis (F34) were older, had lower platelet counts, higher α -fetoprotein, alanine aminotransferase (AST) levels, had higher proportion of diabetes, rs8099917 non-TT genotype carriage, APRI & FIB-4 level. Logistic regression analysis revealed that factors associated with advanced liver fibrosis included Age, diabetes, α -fetoprotein, platelet count, rs8099917 non-TT genotype carriage. While patients were divided based on their viral genotype. Factors independently associated with advanced liver fibrosis in patients with HCV-1 infection included diabetes, α -fetoprotein, platelet count, rs8099917 non-TT genotype carriage. On other hand, factors independently associated with advanced liver fibrosis in patients with HCV- non 1 infection included Age, platelet count.

Conclusions: Unfavorable IL-28B genotype was associated with advanced liver disease. The genetic effect was restricted to patients with HCV-1 infection.

P-0053

Elasticity modulus by real-time color mapping shear wave elastography in SVR patients of CH-C

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Purpose: Some patients develop hepatocellular carcinoma (HCC) after sustained virological response (SVR) to interferon therapy for chronic hepatitis C (CH-C). The aim of this study was to examine the linkage between liver elasticity and presence/absence of HCC in patients after SVR.

Methods: We enrolled 42 patients who underwent real-time color mapping shear wave elastography (SWE) after SVR to interferon therapy for CH-C. Of 42 patients, 6 had HCC and 36 patients did not. We compared the elasticity modulus and other clinical parameters between patients with and without HCC retrospectively.

Results: Elasticity modulus measured by SWE, age, and serum albumin were significantly different between patients with and without HCC. Age, Fibrosis-4 index, serum gamma-globulin, total protein, and albumin levels were significantly correlated with the elasticity modulus. Areas under receiver operating characteristic curves of elasticity modulus, gamma-globulin, and age for the presence of HCC were 0.963, 0.888, and 0.778, respectively. In patients with an elasticity modulus 6.5 kPa and over, both sensitivity and specificity for the presence of HCC were 83.3 %.

Conclusion: The study demonstrated the close linkage between elasticity modulus by SWE and the presence of HCC in patients after SVR.

P-0054

A randomized study of the PegIFN-based regimen with simeprevir for chronic hepatitis C

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Background: Combination therapy with peginterferon (PegIFN) α 2a or α 2b, ribavirin (RBV), and simeprevir (SMV), 2nd generation protease inhibitor, has been used for the patients with HCV genotype 1 in Japan. It is unclear that which type of PegIFN is better to treat these patients.

Methods: Eighty nine patients were enrolled in this study, and randomly divided into 2 groups; PegIFN α 2a group or PegIFN α 2b group.

PegIFN α 2a were administered to 41 patients, whereas PegIFN α 2b, to 45 patients. We compared sustained virological response 24 weeks post-treatment (SVR24), SVR12, and the discontinuation rate. The significant contributors to SVR12 were analyzed using multiple logistic regression.

Results: The median age was 63 years. The proportion of minor alleles (TG or GG) of IL28B SNP (*rs8099917*) is 33.4 %. The SVR24 rate was 88.3 % (40/48). No significant difference of SVR 24 rate was observed between PegIFN α 2a and PegIFN α 2b (88.0 % (22/25) and 78.3 % (18/23)), respectively, $P = 0.303$). The discontinuation rate was similar between both groups (PegIFN α 2a: 16.1 % (5/31) and PegIFN α 2b: 14.7 % (5/34), $P = 0.853$). Multivariate analysis identified two pretreatment factors associated with SVR12; response to prior treatment (non-responder, odds ratio 0.050) and IL28B (TG or GG genotype, odds ratio 0.036). SVR12 rate of the patients with non-responder to prior treatment was 44.4 %, and that of the patients having minor alleles was 65.0 %.

Conclusion: No significant difference of SVR24 is observed between PegIFN α 2a and PegIFN α 2b group. In case of IFN-based regimen for the patients with HCV genotype 1, the difference of PegIFN would not influence SVR.

P-0055

Age and gender influences on chronic hepatitis C (CHC) patients treated with PegINF and ribavirin

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Introduction: It is believed that female gender and age <40–45 years/old have better result in CHC treatment with standard scheme, although many studies are contradictory.

Aim: To evaluate the influence of age and gender on treatment response in CHC patients in Albania.

Patients and methods: The study analyzed 151 patients diagnosed with CHC in UHC “Mother Tereza” service of Gastrohepatology. All the patients were treated with PegINF alfa-2a, 180 μ g s.c/week and weight-based ribavirin 800–1200 mg/day. 82 patients were males (54.3 %) and 69 (45.7 %) females. Median age \pm STD was 45.5 \pm 13.3 years/old. The overall SVR rate was 61.5 % (93 pt). We assessed the influence of age <45 and \geq 45 years/old in relation to gender on treatment response. Data were analyzed statistically by Chi-square test. $p < 0.05$ is considered significant.

Results: In general patients aged < 45 years/old had a SVR rates of 59.2 % and those \geq 45 years/old 64 % without significant differences between groups (Chi-square = 0.366, $df = 1$, $p = 0.545$). Female patients had a SVR rate of 63.7 % and males 59.7 % without significant differences (Chi-square = 0.255, $df = 1$, $p = 0.614$). The SVR rates of females <45 years /old, females \geq 45 years/old, males <45 years/old and males \geq 45 years/old were respectively 66.7, 61.9, 55, 66.7. Females <45 years/old had higher SVR rates than males of the same age (66.7 vs 55 %) and older males \geq 45 years/old had higher SVR rates than younger males(66.7 vs 55 %) but in all cases without significant differences.

Conclusions: Age and gender are not significant predictors of treatment response in CHC patients in Albania.

P-0056

Boceprevir-based triple therapy to rescue HCV genotype 1/HBV dually infected in Taiwan

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Introduction: Recent study showed that the risk of HCC incidence is even higher among HBV/HCV co-infected persons than those with HBV or HCV mono-infection. Previous studies showed that the PEG-IFN/RBV has been effective in the treatment of HCV-dominant, treatment-naïve patients with HCV/HBV dual infections. The aim of this study is to explore the safety and efficacy of boceprevir-based triple therapy to rescue HCV genotype 1/HBV dually infected Taiwanese patients refractory to peginterferon plus ribavirin combination therapy.

Materials and methods: We enrolled 12 eligible patients who agree to join this clinical trial from Kaohsiung Medical University Hospital (KMUH) and National Taiwan University Hospital (NTUH) from March 2014 to December 2014. These 12 patients were classified according to if patients suffered from liver cirrhosis and the response of previous PEG-INF/RBV therapy (relapser, partial responder, and null responder.)

Result: 7 relapsers and 5 null responders were among these 12 subjects. 8 male and 10 HCV genotype 1b subjects were enrolled in this study. No event of death happened in this study, and 2 SEA were noted. Anemia (1/6), but no neutropenia and thrombocytopenia were also noted. Until now 1 of 3 relapser reach to the SVR 12, and percentage of undetectable HCV RNA more than 70 % during period of therapy regimen.

Conclusions: Even this study is in progress, we think that from the preliminary data, boceprevir-based triple therapy to rescue HCV genotype 1/HBV dually infected patients refractory to peginterferon plus ribavirin combination therapy is effective and under consideration.

P-0057

Cases of chronic hepatitis C treatment in hemodialysis patients

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Aim: HCV seroprevalence in chronic hemodialysis patients in our country is 9.8 %. We report our experience with HCV-related liver disease on long-term dialysis.

Methods: Case1: A 36-year-old hemodialysis patient was diagnosed with CHC (Chronic Hepatitis C) followed for 5 years. He had received IFN alpha2a and ribavirin along 1 year. When he admitted to our department, he was using PEG-IFN alpha2a (180 mcg/week) which started 3 months ago. At the end of treatment (at 48th week) breakthrough detected. Case2: A 43-year-old male hemodialysis was followed with diagnosis of CHC for 10 years. We began PEG-IFN

alpha2a 135 mcg/week to him. Treatment could be given 45 micrograms per a week. Viral load had not decreased at 12th week. Treatment was discontinued. Case3: A 39-year-old male hemodialysis patient was diagnosed with CHC. Two years ago, thymus alpha and IFN-2a (3 MU \times 3/week) were used along 5 months. We decided to proceed with a second antiviral treatment using PEG-IFN alpha2a 135 mcgr/week. Relapse occurred at 3 months after treatment. Case4: A 54-year-old hemodialysis patient was diagnosed with CHC 1 year ago. We began PEG-IFN alpha2a 135 mcg/week. At 14th week, there was no response to the treatment.

Conclusion: In CRF patients, treatment with interferon, can not obtain a complete response. Simeprevir, daclatasvir, and the combination of ritonavir boosted paritaprevir, ombitasvir and dasabuvir can be used in patients with severe renal disease. In our country, we need to gain experience with this group of drugs.

Case 1	AST	ALT	wbc	Hgb	Plt	HCV RNA(copy/mL)
0.week	25	6	4800	10,5	106.000	3.000.000
12. week	29	7	4300	10,4	99.000	1.500
24. week	27	7	3600	10,2	128.000	
48. week	19	13	3000	7,1	119.000	286.000
Case 2	AST	ALT	wbc	Hgb	plt	HCV RNA
0.week	17	21	3130	11,4	71.000	520.000
4. week	24	19	4100	13,1	131.000	430.000
12. week	16	12	2790	13	96.000	280.000
Case 3	AST	ALT	wbc	Hgb	plt	HCV RNA
0. week	48	84	4790	13,4	112.000	2.600.000
12. week	27	25	3290	11	109.000	1.563
24. week	29	24	3000	12,5	162.000	+
48. week	18	14	2500	9,7	118.000	Negative
12. week after treatment	46	141	3800	13,7	86.000	27.000.000
Case 4	AST	ALT	wbc	Hgb	plt	HCV RNA
0. week	99	135	4400	13,3	207.000	100.000
4. week	37	18	2830	12,4	142.000	130.0000
12. week	53	50	3160	11,8	102.000	33.000

P-0058

Change in serum levels of M2BPGi in patients with HCV during the treatment of Peg-IFN and RBV

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The sustained virological response (SVR) rate in the patients with HCV has currently reached to 90 % by the progression of anti-viral therapy. However, several reports demonstrated that hepatocellular carcinoma develops even in the patients with SVR. It is widely accepted that liver fibrosis plays a pivotal role in hepatocellular carcinogenesis. Thus, an accurate staging for liver fibrosis is necessary to improve long-term prognosis of hepatitis C patients. Recently, Mac-2 binding protein glycosylation isomer (M2BPGi) was identified as a novel hepatic fibrosis marker. In the present study, we compared the value of M2BPGi in serum before and after the anti-viral therapy in hepatitis C patients. Results of precision, dilution linearity and limit of detect were good performance. The value of M2BPGi in patients with F2, F3, or F4 stagings was significantly higher than that in F1 staging. Moreover, the value of M2BPGi significantly decreased after the treatment with pegylated interferon plus ribavirin similarly to other liver fibrosis-related markers. In addition, the value of M2BPGi in patients with SVR was significantly decreased after the anti-viral therapy (P < 0.0001). The reduction of M2BPGi in SVR patients was thought to reflect the improvement of liver fibrosis, in conjunction with the reduction of viral load, after the treatment. In conclusion, the measurement of M2BPGi in serum might be useful in monitoring the improvement of liver fibrosis by anti-viral therapy.

P-0059

Biochemical and hematological profile at baseline predicts non-SVR in chronic hepatitis C treatment

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Introduction: A number of host and viral factors influence response to therapy among chronic hepatitis C (CHC) patients treated with standard scheme. Prediction of non response in clinical practice from routine blood tests is important.

Aim: To evaluate the impact of pre treatment levels of biochemical and hematological parameters in CHC treatment response.

Patients and methods: A total of 151 patients, diagnosed with CHC in UHC “Mother Tereza” service of Gastrohepatology were included in this study. All the patients were treated with PegINF alfa-2a (180 μ g s.c/week) and weight based RBV 800–1200 mg/day. The median baseline levels \pm STD of total cholesterol (TC), TGC, Fast blood glucose (FBG), GGT, AST, ALT, WBC, HB, and PLT in both SVR and Non-SVR group were compared. Data were analyzed statistically by T test. P < 0.05 is considered significant.

Results: From all patients 93 had SVR (61.5 %) and 58 non-SVR (38.5 %) regardless the genotype. The median baseline levels \pm STD of parameters in Non-SVR versus SVR group for TC (mg/dl), TGC (mg/dl), FBG (mg/dl), GGT (U/l), AST (U/l), ALT (U/l), WBC/mm³, HB (g/dl), PLT/mm³ were respectively: 151.71 \pm 31.55 vs 174.30 \pm 37.46 (p = 0.005); 115.14 \pm 68.21 vs 106.48 \pm 52.10 (p = 0.5); 107.32 \pm 41.72 vs 98.72 \pm 30.77 (p = 0.5); 82.90 \pm 62.74 vs 56.56 \pm 57.55 (p = 0.04); 62.8/ml \pm 33.57 vs 66.3 \pm 62.08 (p = 0.35), 77.8 \pm 49.14 vs 114.4 \pm 136.4 (p = 0.079); 6143.33 \pm 1725.42 vs 6462.35 \pm 1718.63 (p = 0.2); 13.88 \pm 1.62 vs 13.79 \pm 1.60 (p = 0.7); 179,145.37 \pm 64,504.51 vs 219,827.06 \pm 80,966.55 (p = 0.002).

Significant statistical differences in baseline TC, GGT and PLT were found between Non-SVR and SVR groups.

Conclusions: In routine clinical practice significant negative baseline predictors of SVR were low level of TC, low level of PLT and high level of GGT.

P-0060

The role of ubiquitin specific protease USP18 in interferon resistance of HCV

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Hepatitis C Virus (HCV) infects 150 million people worldwide. No prognostic tools are available and the molecular mechanisms of the interferon resistance remain illusive. Previous work from our lab identified 18 differentially expressed hepatic genes whose expression levels prior to treatment predict whether a given patient will respond to IFN-based therapy or not (with an accuracy of 96 %). Of these 18 genes 11 are interferon stimulated genes (ISGs) whose expression levels are higher in treatment non-responder livers. Especially the activation of the ubiquitin-like ISG15/USP18 pathway plays an important role in interferon resistance and treatment failure. To study the effect of USP18 on HCV replication and Jak/STAT signaling pathway as well as other innate immune response pathways. We screened 11 cellular proteins which could interact with ubiquitin specific peptidase 18 (USP18) by immunoprecipitation and mass spectrometry. The Co-immunoprecipitation (Co-IP) assay were employed to further confirming the interaction between USP18 and NME/NM23 nucleoside diphosphate kinase 1 (NME1) or inhibitor of kappa light polypeptide gene enhancer in B-cells kinase gamma (IKBKG). Next we will study how USP18 affects HCV replication and how the virus evades the innate immune attack and counteracts the IFN anti-viral activity leading to treatment failure and viral persistence. This study will shed light on how HCV exploits the host innate immune response to benefit its own replication and to blunt the interferon anti-viral activity.

P-0061

Efficacy and safety of SMV-based triple therapy for patients aged 66 years and older with CHC

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Background: The majority of patients with chronic HCV infection are older in Japan than in the United States and Europe. Although a HCV protease inhibitor, simeprevir (SMV)-based triple therapy combined with PegIFN α plus ribavirin (RBV) has resulted in an improved SVR rate, there are only a few reports describing the differences in factors predictive of SVR in older and younger patients.

Aims: To evaluate the efficacy and safety of SMV-based triple therapy in older patients, specifically aged 66 years and over.

Methods: This prospective study enrolled 85 genotype 1b Japanese patients with CHC who received 12 weeks of triple therapy followed by a 12-week dual therapy. The patients were categorized according to age: an older group (42 patients aged >65) and a younger group (43 patients aged <66).

Results: No significant differences in the SVR were found between the older (82.1 %) and younger (81.0 %) groups. The SVR rates for patients with the IL28B (rs8099917) TT allele (100 and 88.2 % for the older and younger groups) were significantly higher than for patients with the IL28B TG/GG allele (64.7 and 77.8 %, respectively). A multivariate analysis identified the IL28B TT allele as an independent factor associated with the SVR. Adverse events resulted in treatment discontinuation by 14.3 and 7.0 % in the older and younger groups, respectively.

Conclusions: SMV-based triple therapy can be successfully used to treat patients aged 66 years and over with genotype 1b CHC. IL28B genotyping indicates the potential to achieve an SVR in these difficult-to-treat older patients.

P-0062

Efficacy of second-generation protease inhibitor-based triple therapy for HCV genotype 1b patients

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Aims: The introduction of second-generation NS3/4A protease inhibitors has contributed to a decrease in the incidence of adverse effects compared to telaprevir and boceprevir. The aim of this study was to

evaluate the efficacy and safety of simeprevir or vaniprevir in combination with peginterferon and ribavirin (PR) for non-cirrhotic patients with chronic hepatitis C virus (HCV) genotype 1b infection. **Methods:** This prospective, multicenter study consisted of 368 Japanese HCV genotype 1b patients treated in a clinical setting (simeprevir 337 and vaniprevir 31). All patients received a combination treatment of simeprevir (100 mg QD) or vaniprevir (600 mg BID) and PR for 12 weeks, followed by an additional 12 weeks of PR alone.

Results: The sustained virological response rates, by intention-to-treat analysis, 12 weeks after the end of treatment (SVR12) were 82.2 % (277/337) and 85.7 % (18/21, the results of the 10 others are pending) for simeprevir- and vaniprevir-based triple therapy, respectively. In the simeprevir group, prior treatment response to PR (treatment-naïve 87.6 %, relapse 89.5 %, partial response 60.9 %, and null response 26.1 %) and interleukin (IL)28B genotype (rs8099917) (TT 90.8 %, TG/GG 61.2 %) were independently associated with SVR12. Severe anemia (hemoglobin <9.0 g/dL) was observed for 90 (26.7 %) and 5 (16.1 %) patients in the simeprevir and vaniprevir groups, respectively. Overall, 38 (11.3 %) and 4 (12.9 %) patients in the respective groups discontinued treatment, mainly due to fatigue (simeprevir) and nausea (vaniprevir).

Conclusions: Triple therapy with simeprevir or vaniprevir was well tolerated and achieved high SVR rates for treatment-naïve and prior relapse patients with the favorable IL28B genotype.

P-0063

Factors that predict pretreatment and on-treatment SVR in CHC C patients treated with dual therapy

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Background: Despite emergence of DAA, dual therapy of Peg-IFN-alpha2 and ribavirin is still widely used as the standard treatment for hepatitis C, especially in Indonesia. Due to its high cost and side effects, the possibility of pretreatment and on-treatment prediction would be advantageous. The aim of this study is to formulate prediction of SVR and avoid unnecessary treatment in hepatitis C patients.

Methods: Case control study was conducted to evaluate risk factor in achieving SVR. All data including age, sex, HCV RNA, platelet, SNP IL-28B, RVR, and viral genotype were analyzed using multivariate backward stepwise. The quality of risk equation was measured using calibration and discrimination parametric according to Hosmer and Lemeshow test and receiver operator characteristics methods, respectively.

Results: Total of 123 naive chronic hepatitis C patients who have completed treatment with Peg-IFN-alpha2 and ribavirin were included in this study. While SNP IL-28B and platelets level were found to be significant pretreatment factors, RVR was the only significant on-treatment factor. Risk factor equation for SVR was formulated into $p = 1/(1 + e^{-y})$; $e = 2.7$, $y = -2.498 + 2.652$ (SNP IL-28B) + 2.029 (thrombocytes) for pretreatment and $y = -0.223 + 2.621$ (RVR) for on-treatment. The use of risk factor equation for predicting SVR was able to avoid unnecessary therapy in 16-29 % of cases.

Conclusion: Even in The era of DAA, treatment with dual Therapy still the SOC for chronic Hepatitis C infection in Indonesia. Application of pretreatment and on-treatment equation will reduce the probability of non-SVR. This will affect the total treatment cost, risk of side effects and drugs interaction.

P-0064

FIB-4 can predict HCC development in CHC patients with SVR to interferon-based combination therapy

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Background: Interferon-based combination therapy can prevent the development of hepatocellular carcinoma (HCC), especially in CHC patients with sustained virologic response (SVR). However, some patients with SVR remain at risk of HCC. We evaluated the role of aspartate aminotransferase to platelet ratio (APRI) and fibrosis-4 (FIB-4) index to predict the development of HCC.

Methods: A total of 209 patients with SVR following interferon-based combination therapy were enrolled retrospectively.

Results: HCC developed in 9 patients during 5.5 years of mean follow-up. The cumulative incidences of HCC at 1, 5, and 10 years were 0, 3.5, and 9.2 %, respectively. Univariate analysis showed that risk factors of HCC development were high APRI (>1.5), FIB-4 (>4.5), age (>60 years), low platelet and cirrhosis. The cumulative incidences of HCC were higher in patients with high APRI than in those with low APRI (0, 8.3% and 19.2 vs. 0 %, 0.8 and 0.8 % at 1-, 5-, and 10-years) ($p = 0.002$). They were higher in patients high FIB-4 than in those with low FIB-4 (0, 10.5 %, and 24.5 vs. 0 %, 0.7 and 0.7 % at 1-, 5-, and 10-years) ($p = 0.0001$). High FIB-4 (RR 13.1; 95 % CI 1.3–99.3) and cirrhosis (RR 4.5; 95 % CI 1.1–17.8) were independent predictor of HCC. The estimated annual incidence of HCC in patients with high FIB-4 and APRI was 2.4 and 4.3 %.

Conclusion: HCC risk is high in patients with high FIB-4 or cirrhosis. HCC surveillance should be performed in these patients even after SVR.

P-0065

Genome-wide association study on genetic variants associated with interferon-induced depression

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The interferon-based therapy is known to cause depression that frequently interrupts treatment. To identify genetic variants associated with interferon-induced depression in chronic hepatitis C (CHC) patients, we conducted a genome-wide association study (GWAS). We enrolled total 384 Japanese CHC patients receiving interferon-based therapy in multicenter prospective study and stratified them into two groups: case (BDI-II >20) and control (BDI-II <20) according to the Beck Depression Inventory II (BDI-II) score during interferon-based therapy. At the GWAS stage, we genotyped 224 subjects with case (n = 45) and control (n = 179). Based on the results, we selected 42 candidate single nucleotide polymorphisms (SNPs) and performed replication analysis in an independent set of 160 subjects with case (n = 40) and control (n = 120). The SNP rs1863918 in strong linkage disequilibrium with SNPs located around the *ZNF354C* gene on chromosome 5, showed a significant association when the results of GWAS and replication were combined (OR = 2.55, $P = 7.89 \times 10^{-8}$), suggesting that rs1863918 TT/TG genotypes were associated with interferon-induced depression. Logistic regression analysis showed that rs1863918 TT/TG genotypes, a history of depression, and younger age were independent predictive factors for interferon-induced depression. Furthermore, the proportion of patients with a history of depression was significantly higher in patients with rs1863918 TT/TG (13.1 %) than GG (4.9 %) ($P = 0.007$). Interestingly, western blotting and immunohistochemistry showed that the *ZNF354C* protein was highly expressed in the hippocampus of mice implicated in the pathology of depression. In conclusion, we identified a SNP significantly associated with interferon-induced depression, and revealed that the candidate gene was highly expressed in the hippocampus of mice.

P-0066

Cost-effectiveness of NEUTRINO regimen for CHC GT1b in China: a real life study

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Background and aims: Direct-acting antivirals (DAAs) agents have drastically improved the sustained virologic response (SVR) rates but are very costly. We determine the cost-effectiveness of 12-week SOF + PEG/RBV (“NEUTRINO” regimen) compared to 48 weeks

PEG/RBV standard-of-care (SOC) in chronic hepatitis C (CHC) genotype 1b patients in real-life practice in Chinese.

Methods: A decision analytic Markov model based on the natural history of CHC was developed from the perspective of third-party payer to estimate health outcome in quality adjusted life years (QALYs), lifetime cost of CHC and the incremental cost-effectiveness ratios (ICERs). The model included treatment-naïve and treatment-experienced cohorts with subgroup analysis for those with and without cirrhosis. SVR rates and direct medical costs were from real life data, collected at a single Hong Kong-Beijing liver center. Parameter uncertainty was assessed by one-way sensitivity analysis and probabilistic sensitivity analysis.

Results: SOF + PEG/RBV increased QALYs and costs resulting in ICERs of US\$15,313–90,772 per QALY gained. ICERs for non-cirrhotic patients in treatment-experienced cohort was US\$15,313, which was lower than that for cirrhotic patients. ICERs for treatment-experienced cohort were lower than that for treatment-naïve cohort in any patient. The results were sensitive to post-SVR utility, cost of SOF + PEG/RBV and discount rate. The probability of SOF + PEG/RBV being cost-effective was 0.522 and 0.733 at US\$45,564 (6 times GDP per capita) in treatment-naïve and experienced patients respectively.

Conclusion: Due to the high cost of treatment, the regimen is not cost-effective across all patient groups.

P-0067

HCV quasispecies complexity and diversity of Indonesian chronic HCV patients treated with PegIFN/RBV

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The current standard treatment for patients with chronic hepatitis C consists of pegylated alpha interferon in combination with the nucleoside analogue ribavirin (peg-IFN/RBV) for half- or a year-round and leads to a sustained virological response (SVR). The majority of patients with chronic hepatitis C fail to respond to antiviral therapy and the genetic basis of this resistance is unknown. Within an infected individual, HCV circulates as a set of closely related variants referred to collectively as quasispecies. The quasispecies nature of HCV may have important implications concerning viral persistence, pathogenicity, and treatment response. This study was aimed to characterize HCV quasispecies diversity and complexity also its association with the peg-IFN/RBV treatment response. To evaluate whether the quasispecies pre-treatment composition of HCV is related to responsiveness of therapy, a total of 680 sequence clones spanning HCV Core and NS5A region were generated from pre-treatment plasma samples obtained from 17 chronic hepatitis C patients which showed Rapid Viral Response (8 patients) and Non Rapid Viral Response (9 patients). Detailed quasispecies diversity and complexity were then analyzed. The NRVR group showed higher genetic complexity than the RVR group in Core and NS5A region, albeit statistically not significant. Moreover, no statistically significant differences were observed when comparing quasispecies diversity and dN/dS ratio in the NRVR and RVR group in HCV Core and NS5A region. Although a difference of quasispecies diversity and complexity was observed between RVR and NRVR group, it may

need further analysis to evaluate the association of quasispecies with early treatment response.

P-0068

HCV reactivation and HCC development after achievement of HCV RNA negative by Peg-IFN/Rbv therapy

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Background and aim: The sensitivity of detection system for HCV RNA is important to diagnose the sustained viral response (SVR). Recently, we experienced a case of reactivation of hepatitis C after diagnosis with SVR.

Patient and methods: The patient was treated by pegylated interferon (Peg-IFN) and ribavirin, and achieved HCV RNA negative and diagnosed with SVR by HCV RNA qualitative test. After 9 years, he developed chronic hepatitis with HCV and hepatocellular carcinoma. HCV strains of before and after treatment were isolated and sequenced. The serum samples of several points at the diagnosis of SVR were re-assessed by highly sensitive HCV RNA detection kit (real-time PCR).

Results: HCV strains isolated before and after treatment were closely related and considered to be progenies from same origin. By real-time PCR, HCV RNA was detected at the point of 24 weeks after treatment. These results suggested that HCV was not eradicated by Peg-IFN and ribavirin. The persistence of HCV infection in this patient seemed to be involved with development of hepatocellular carcinoma.

Conclusion: This case indicates that the long-term surveillance for HCV RNA with highly sensitive detection method is indispensable even after achievement of SVR.

P-0069

Impact of HCV kinetics differs by the type of real-time assay used to monitor triple therapy

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Aims: Repeated measurement of the HCV RNA level is essential for properly monitoring treatment efficacy. The study was done to compare the ability of two HCV RNA assays to evaluate the impact of hepatitis C virus (HCV) kinetics on the outcome of patients treated

with triple therapy that included the NS3/4A protease inhibitors telaprevir and simeprevir.

Methods: This prospective, observational study consisted of 171 Japanese patients infected with HCV genotype 1. All 2667 serum samples taken during treatment were tested with both the COBAS AmpliPrep/COBAS TaqMan (CAP/CTM) HCV Test v2.0 and the Abbott RealTime (ART) HCV Test.

Results: Of the 917 samples undetectable for HCV RNA by the CAP/CTM assay from the first 8 weeks of treatment, 343 (37.4 %) (312 detectable/lower limitation of quantification and 31 quantifiable) were positive by the ART assay. The time HCV RNA became undetectable by ART was associated with SVR for difficult-to-treat group, such as patients with advanced fibrosis, IL28B TG/GG genotype (rs8099917), and prior non-responders. In contrast, for non-difficult-to-treat group, almost all of the late responders by ART achieved SVR, unlike by CAP/CTM. Despite HCV RNA being once undetectable by ART, 50 (33.1 %) patients experienced the reappearance of residual HCV RNA. However, this phenomenon by week 12 was not related to treatment failure.

Conclusions: The ART assay was superior to the CAP/CTM assay in the detection of HCV RNA of very low viremia. This advantage is very important for predicting SVR by difficult-to-treat patients and treatment failure.

P-0070

May triple therapy still be an alternative to standard therapy in patients with chronic hepatitis C?

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Background: Recent developments in hepatitis C therapy resulted in nearly 100 % sustained virological response rates but unfortunately these modern therapies are not reimbursed in some countries including Turkey. Triple therapies of boceprevir (BOC) and telaprevir (TVR) are still in use especially for the patients who need urgent therapy.

Aim: To evaluate the effectiveness of triple therapy and compare it with standard therapy.

Material and methods: Of the 224 patients, 81 were in triple therapy group (TTG) [ribavirin (1200 mg/ day in patients >75 kg and 1000 mg <75 kg) + pegylated interferon + BOC/TVR] and 143 were in standard therapy group (STG) (ribavirin + pegylated interferon). In both groups therapies were given under response guidance. HCV-RNA negativity at the end of 24 weeks of follow up was accepted as sustained virological response (SVR24).

Results: TTG and STG were similar in terms of mean age (57.5 ± 7.9 and 55.6 ± 10.5), gender (M/F 27/54 and 47/96), body mass index (27.7 ± 4.2 and 27.4 ± 4.9), initial HCV-RNA level, genotype (1b and 1a), initial transaminase level and platelet level ($p > 0.05$). TTG included more cirrhotic patients (26 vs 18). SVR24 was 63 % in TTG and 52 % in STG ($p > 0.05$). Rate of hematological and dermatological adverse events, therapy discontinuation due to adverse event and transfusion were more frequent in TTG ($p < 0.05$).

Conclusion: Triple therapy is not statistically superior to standard treatment in terms of efficacy and side effects and modern therapies with higher efficacy and lower side effects should replace previous therapies urgently.

P-0071

Outcomes of SOF, RIBA & PEG-INF therapy for treatment of chronic HEP-C G3 treatment naive patients**Muzzaffar Gill, Sidra Gill, Nosheen Yousaf, Ammara Nawaz, Faiza Irfan**

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Background: Sovaldi, Ribavirin and PEG-Interferon is gold standard therapy for chronic HEP-C patients. It is recommended both for treatment naive and experienced patients. We evaluated the safety and efficacy of this combination in naive chronic HEP-C G3 patients.

Methods: We prospectively enrolled 50 chronic HEP-C G3 treatment naive patients. Patients with de-compensated cirrhosis, platelets count <100, portal vein size >13 mm on ultrasound in size were excluded. Eligible patients were enrolled to receive Sovaldi 400 mg daily, Ribavirin 10 mg/kg body weight and Pegylated interferon 1.5 UCG/KG body weight for 12 weeks. Patients were seen in outpatient 4-weekly basis. They had baseline CBC, TSH, Blood Sugar, HCV Genotype, and HCVPCR quantitative. Each patient had CBC at every 4 weeks. RVR at treatment week 4, and ETR (End of Treatment) at weeks 12 were defined by HCV-RNA <25 IU/mL.

Results: This is our ongoing study we are presenting interim analysis of 50 patients who have completed 12 weeks of treatment. Out of 50 patients 75 % were male, 25 % were female with median age 48 years. 48 patients out of 100 (96 %) were PCR negative at week 4 and 96 % were PCR negative at week 12 respectively. Two patients did not achieve RVR, and they did not achieve ETR either. The most common AEs were nausea, fatigue, dizziness.

Conclusions: Sofosbuvir + Ribavirin + PEG INF in HCV-G3 infected patients is very effective treatment. Most patients achieved rapid Virologic response without any major side effects. These patients are under regular review to monitor SVR.

P-0072

Paired FIB-4 score in successfully treated chronic hepatitis C with pegylated interferon & ribavirin**Muhammad Imran, Karim Kammeruddin, Nida Sajid, Amjad Iqbal**

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Background: The FIB-4 Score, is a simple formula to predict liver fibrosis based on the standard biochemical/haematological values i.e AST, ALT, Platelet count and age. This Score lower than 1.45 has a Negative Predictive Value (NPV) of 95 % for significant fibrosis i.e F3–F4 while, a Score of greater than 3.25 has a PPV more than 80 % for F3–F4. So an improvement of FIB-4 Score is a possible indicator of change in liver fibrosis.

Objective: To observe the difference in Paired FIB-4 Score in Successfully treated Chronic Hepatitis C (CHC) patients with Pegylated Interferon & Ribavirin based therapy.

Method: An observational study, in patients who were mono-infected, compensated CHC patients, treated with Pegylated Interferon

& Ribavirin for 24 to 48 weeks (according to genotype) in a tertiary care hospital (i.e Baqai Medical University Hospital Nazimabad) from Jan 2010 to March 2015 and has successfully achieved ETR (n = 58) i.e HCV RNA Not detected at ETR. The FIB-4 Score was applied in a paired manner i.e. before (at beginning) and at the End of treatment evaluation (ETR).

Results: Males 24 (41.4 %) with mean age 38.8 (22–60 years) and Females 34 (58.6 %) with mean age 43.6 (31–60 year). FIB-4 scores at beginning/At ETR evaluation less than 1.45 30 (51.7 %)/36 (62.1 %) 1.45–3.25 16 (27.6 %)/12 (20.7 %) More than 3.25 12 (20.7 %)/10 (17.2 %).

Conclusion: Definite positive trend of Change in FIB-4 Score was observed with pegylated interferon/ribavirin based treatment even as early as ETR in successfully treated Chronic Hepatitis C patients. Keywords: FIB-4 Score, Chronic Hepatitis C & Pegylated interferon.

FIB-4 Scores	At Beginning	At ETR Evaluation
Less than 1.45	30 (51.7%)	36 (62.1%)
1.45-3.25	16 (27.6%)	12 (20.7%)
More than 3.25	12 (20.7%)	10 (17.2%)

P-0073

Peg-interferon and ribavirin in naive patients with mild to moderate genotype1b chronic hepatitis C**Hurrem Bodur, Esragul Akinci, Aliye Bastug, Ayse But, Halide Arslaner, Pinar Onguru, Meltem Arzu Yetkin**

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Introduction: In recent years, some highly effective new therapeutic agents were introduced in to the market for treatment of chronic hepatitis C (CHC). However, these agents are very expensive and having these drugs may be difficult in the areas with limited sources. This study was performed to suggest the effectiveness of conventional treatment regimen in naive patients with mild to moderate genotype-1b CHC.

Methods: Naive patients with mild to moderate genotype-1b CHC (fibrosis stage lower than 4) who were treated with pegilated-interferon and ribavirin combination for 48 weeks included in to the study.

Results: Thirty patients measured up the study inclusion criteria. Laboratory parameters were as follows: Mean ALT: 56.8 ± 43.9 IU/L, median HCV-RNA: 971,000 copies/ml (14,800–80,000,000), mean fibrosis score: 1.6 ± 1, mean histological activation index: 6.1 ± 3. In the follow ups, 17 (56.7 %) patients had rapid virological response, 26 (86.7 %) patients had early virological response and 27 (90 %) patients had virological response at the end of the treatment. Sustained virological response (SVR) was gained in 23 (76.7 %) patients and all of these patients had undetectable HCV-RNA at the end of the 1st year after completion of the treatment.

Conclusion: Response to conventional treatment with pegilated-interferon and ribavirin is high (76.7 %) in naive patients with mild to moderate genotype-1 CHC. This regimen yet can be a good option for treatment of these patients living in areas where new therapeutic agents are unavailable.

P-0074

Peginterferon plus ribavirin for HIV-infected patients with acute or chronic HCV infection in Taiwan

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Background: Data are limited on the effectiveness and safety of peginterferon plus ribavirin in HIV-infected Asian patients with acute or chronic HCV infection.

Methods: HIV-infected Taiwanese patients with acute HCV infection received peginterferon plus weight-based ribavirin for 24 weeks ($n = 24$), and those with chronic HCV genotype 1 or 6 (HCV-1/6) and HCV genotype 2 or 3 (HCV-2/3) infection received response-guided therapy for 12–72 and 24–48 weeks, respectively ($n = 92$). The primary endpoint was sustained virologic response (SVR), defined as undetectable HCV RNA 24 weeks off-therapy.

Results: The SVR rates were 83 and 72 % in patients with acute and chronic HCV infection ($p = 0.30$), and 68 and 72 % in patients with chronic HCV-1/6 and HCV-2/3 infection ($p = 0.48$), respectively. While no factors predicted SVR in acute HCV and chronic HCV-2/3 infection, age (odds ratio [OR] per 1-year increase: 0.88, 95 % confidence interval [CI]: 0.78–0.99, $p = 0.04$), HCV RNA (OR per 1-log₁₀ increase: 0.18, 95 % CI: 0.03–0.98, $p = 0.03$), IL28B genotype (OR: 5.52, 95 % CI: 1.55–12.2, $p = 0.02$), and RVR (OR: 9.62, 95 % CI: 3.89–15.3, $p = 0.007$) predicted SVR in chronic HCV-1/6 infection. Conclusion, the SVR rates of peginterferon plus ribavirin for 24 weeks and for response-guided 12–72 weeks are satisfactory in HIV-infected Taiwanese patients with acute and chronic HCV infection.

P-0075

Pegylated interferon in HCV genotype 3 relapsers to conventional interferon in Pakistani population

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Background: Estimated prevalence of Hepatitis C in Pakistan is 5 % of which 78 % are Genotype 3, in which Response to conventional interferon is reported to be 70 %.

Objective: To determine the efficacy of pegylated interferon 20 kDa (Unipeg ®) plus ribavirin (Ribazole ®) in HCV genotype 3 patients who relapsed to conventional interferon.

Methods: 20 patients were enrolled. Pegylated interferon alfa 2a 20 kDa 180 µg weekly with ribavirin, were administered for a period of 24 weeks. Virological Responses were measured by Qualitative HCV RNA at weeks 4, 12, 24 and 48 to see Rapid Virological Response (RVR), Early Virological Response (EVR), End of Treatment (ETR) and Sustained Virological Response (SVR), respectively.

Results: There were 20 patients of which 2 are still receiving therapy. Out of intent-to-treat patients, 15 have completed 24 weeks therapy 12 are male mean age 38.5 (± 7.62) years.; RVR was achieved in 7 (39 %) patients while another 8 patients achieved EVR. 1 patient lost to follow up. ETR was achieved in 14 (93 %) patients. Among 14 ETR achieved patients; 9 patients have so far completed post therapy follow up, of which, 8 (89 %) patients have achieved SVR.

Conclusion: Our interim data demonstrates that Pegylated Interferon alfa 2a 20 kDa 180 µg (Unipeg ®) in combination with Ribavirin (Ribazole ®) has shown promising results in treating HCV genotype 3 patients who relapsed to conventional interferon. We recommend use of Pegylated Interferon in Relapsers with Genotype 3 when financial constraints limit use of Oral available options.

P-0076

Problems in treatment of cirrhotic HCV patients in Azerbaijan-retrospective analyze

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Background: The treatment of hepatitis C in Azerbaijan is centralized and is held only in the capital city-Baku, there are very few specialists in the rest of the country. Majority of population doesn't have enough finances or insurance to cover treatment, low public awareness on Hep C danger leads to the state when many people reach an advanced stage before they receive any treatment.

The aim of the study: evaluation of the SVR of treatment in 370 cirrhotic patients with chronic hepatitis C, 40 % of them were with diabetes mellitus or insulin resistance, 8 % with various oncology diseases.

Methods: We reviewed a total 1928 patients with HCV infection, 370 of them (19 %) were patients with advanced cirrhosis (49 % men) and 179 (48 %) of them were treated by PEG IFN and RIB, during 2003–2014 years.

Results: SVR developed in 48 patients (27 %) out of 179 patients received antiviral treatment: 29 patients with genotype 1 (66 %) and 19 patients with genotypes 2 and 3 (34 %). It was determined that SVR developed in patients that had prolonged treatment duration (60–72 weeks for genotype 1 and 28–48 weeks for 2 and 3 genotypes). SVR develop in 38 patients (79 %) with prolonged treatment in comparison with 10 patients (21 %) with standard schemes of treatment. Additional treatment for correction of complications included thrombopoietin, erythropoietin, filgrastim, metformin, antibiotics.

Conclusions: it was determined that SVR was developed in the cirrhotic patients with prolonged treatment (79 %) and with correction of complications of treatment.

P-0077

Randomized trial of two types of PEG-IFN with simeprevir combined therapy for patients with HCV 1b

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Aim: Simeprevir (SMV) is a potent, macrocyclic hepatitis C virus (HCV) non-structural (NS)3/4A protease inhibitor. This prospective study compared the efficacy and safety of SMV in combination with peginterferon alpha-2a + ribavirin (P2aR) and with peginterferon alpha-2b + ribavirin (P2bR) for Japanese patients with HCV genotype 1b infection.

Methods: HCV1b patients were randomly assigned to SMV (100 mg QD) with P2aR for 12 weeks then P2aR alone for 12 or 36 weeks; or SMV with P2bR for 12 weeks then P2bR alone for 12 or 36 weeks. The primary endpoint was sustained virological response 24 wks after completing treatment (SVR24).

Results: 151 patients were randomly assigned to P2aR group (n = 76) and P2bR group (n = 75). Six patients were dropped out. SVR24 was achieved in 55 (75.3 %) of 73 P2aR patients and 55 (76.4 %) of 72 P2bR patients. There was no difference in SVR24 between two groups (p = 0.88). No differences in the proportion of patients who became HCV RNA-negative were detected between the P2aR and P2bR groups (76.7 and 84.7 % at week 4, and 95.9 and 94.4 % at the end of treatment respectively). The two groups had comparable numbers of adverse events, which led to the discontinuation of treatment in 9.6, and 8.3 % of participants in each of the P2aR and P2bR groups.

Conclusions: PegIFN alfa-2a or alfa-2b in combination with SMV + ribavirin therapy showed the same anti-viral effect for patients with chronic hepatitis C. Also, the occurrence of adverse events was the same both type of pegIFNs.

P-0078

Renal abnormalities in patients with chronic hepatitis C and response to therapy

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Introduction: Limited data is available on prevalence of renal abnormalities in patients with chronic hepatitis C (HCV) and effect of therapy on renal functions. We evaluated the prevalence of kidney disease indicators, in chronic HCV patients, and followed up for evaluation renal abnormalities at the end of treatment.

Materials and methods: Forty five chronic HCV treatment naive patients without hepatic decompensation or renal disease were prospectively included from January 2014 to February 2015. All

patients were treated with PegIFN α 2b 1.5 μ g/kg along with ribavirin (800 mg for weight <60 kg and 1000 mg for >60 kg). Patients were investigated with renal parameters and followed up for 6 months and repeat evaluation for renal abnormalities done at end of treatment. Renal abnormalities were defined using kidney disease outcome quality initiative (KDOQI).

Results: 33 were men, with age 31.6 ± 9.9 years, 80 % were infected with genotype 3, whereas 18 % with genotype 1. All the patients were either had no cirrhosis or only Child A cirrhosis. Prevalence of renal abnormality was 18 % at start of treatment vs 11 % at the end of treatment (p = 0.02). Antiviral therapy significantly reduce creatinine level (0.76 vs. 0.62; p = 0.04), increases eGFR (121 vs 135; p = 0.04) and associated with increased serum albumin level (4.02 vs. 4.42; p = 0.001). RVR was found to be associated with absence of renal abnormality (p = 0.01). There were no significant correlation with change in GFR.

Conclusions: Chronic HCV associated with some renal abnormality in absence of clinical features. Treatment of HCV favorably affect renal functions.

P-0079

Resistance analysis of unsuccessfully treated with telaprevir genotype 1 HCV infected patients

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According to newest guidelines each treatment option of genotype 1 HCV infected patients includes at least one directly acting antiviral agent (DAA). The first generation protease inhibitors boceprevir and telaprevir were used in previous years. Despite high sustained virologic response rate there are still some patients who meet criteria of virologic failure. Detection of resistance association mutations can be useful in planning next treatment regimen. In this study we evaluated the frequency of resistant variants to protease inhibitors NS3 in patients unsuccessfully treated with telaprevir in combination with pegylated interferon alpha and ribavirin by ultra-deep pyrosequencing.

Methods: Ten patients treated with telaprevir who met criteria of virologic failure were included in the study. Ultra-deep pyrosequencing was used to analyse NS3 region of HCV genome. 5 patients were examined from the time when treatment failure occurred to 1 year after failure (group 1), next 5 subjects-2 years after end of treatment (group 2).

Results: All patients from group 1 had resistance associated variants (RAVs) to telaprevir (A156T, A156S, V36M, I132V). In group 2 three patients did not have any RAVs in NS3 region, and only one had resistant associated mutation to telaprevir (I132V). The last patient from group 2 had variant associated with resistance to simeprevir (S122G).

Conclusion: (1) It seems that one of the possible reasons of treatment failure is RAVs occurrence. (2) Pyrosequencing can probably be the useful tool for planning the right therapy with DAAs and for monitoring RAVs persistence after stopping treatment with protease inhibitor.

P-0080

Profile of chronic hepatitis C treated with interferon based regimen in Sardjito General Hospital

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Background: The combination of pegylated interferon 2 α : and ribavirin <Peg-IFN2 α :/RBV> still the standard of treatment for chronic hepatitis C <CHC> in Indonesia. Because of its high cost management, the evaluation of HCV-RNA PCR quantitative level was examined only at the beginning, the end of treatment and SVR period. **Methods:** CHC patients who were treated with Peg-IFN2 α :/RBV in Sardjito General Hospital were evaluated for their HCV-RNA PCR quantitative level, dosage of Peg-IFN2 α :/RBV, and the reasons of dosage changes at the end of treatment and 24 weeks afterwards <SVR> for patients who had reached SVR time period.

Results: Twenty six CHC <IFN-naive> patients completed the treatment schedule based on the HCV genotype between January 2013 and Desember 2014. The median age: 61.9 years old with 61.5 % females. HCV genotype 1 46.2 %; genotype 2: 42.3 %; genotype 3: 11.5 % Fibrosis score <METAVIR> based on liver biopsy or Fibroscan were F1 3.8; F2 3.8 %; F3 19.2 %; F4 38.6 % and 34.6 % without METAVIR score data. All patients reached undetectable HCV RNA-PCR at the end of treatment with 57.6 % patients were prescribed with various reduced dose of peg-IFN and or RBV. Five patients <M3 patients with dose reduction> has reached the SVR during the study period.

Conclusion: Reduced dose of Peg-IFN2 α :and/or RBV did not interfere the successful end of treatment results of chronic hepatitis C treatment.

P-0081

Sofosbuvir, PegIFN and ribavirin for 12 weeks in treatment-naive HCV genotype 1b patient

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Introduction: Treatment options for genotype 1b HCV-infected patients are still limited. Sofosbuvir + PegIFN + ribavirin regimen is an effective treatment method in patients with treatment-naive patients with genotype 1b hepatitis C virus infection according to NEUTRINO and ATOMIC studies, and AASLD/IDSA HCV-2014 guideline. In this case, we aimed to evaluate the efficacy of a 12-week SOF + PegIFN/RBV regimen in treatment-naive patients with HCV GT 1 infection.

Case report: We describe a 72-year-old, Turkish male patient with hepatitis C virus genotype 1b infection. Chronic hepatitis C infection was documented in 2001. Laboratory data showed anti-HCV antibody: positive, HCV RNA: 251.633 IU/ml, genotype: 1b, IL28B: CT. Liver biopsy results showed advanced fibrosis (Ishak score A7F4) and fibrotest score: 0.79 in 2014. He treated with sofosbuvir + PegIFN α -2b + ribavirin regimen for 12 weeks. Serum alanine aminotransferase levels normalized and HCV-RNA became negative at the end of the second week and the triple therapy was continued for 12 weeks. We follow the patient for sustained viral response. Evaluation of HCV RNA at the end of the 4th and 8th weeks of therapy revealed negative results. Adverse reactions associated with the triple therapy were mild anemia (Hb = 10.9 g/dL), fatigue, skin dryness and pruritus. There were no serious drug-related adverse events.

Conclusion: No viral breakthrough or relapse were observed. The regimen was safe and effective in patients with genotype 1b HCV infection.

P-0082

Treatment chronic hepatitis C Myanmar patients with sofosbuvir, pegylated interferon and ribavirin

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Pegylated Interferon and Ribavirin combination treatment was the standard of care in Myanmar before the availability of Direct Acting Antivirals (DAAs). However, at the beginning of 2015, one of the DAAs namely generic Sofosbuvir made in India became available in Myanmar. Therefore, Sofosbuvir, Pegylated Interferon and Ribavirin triple drug combination for 12 weeks was used for the treatment of Interferon eligible patients with Chronic Hepatitis C infection. 180 Myanmar patients with Chronic Hepatitis C infection were treated with above mentioned triple drug regimen. Out of 180 patients 83 were male and 97 were female. There were 73 genotype 3 patients, male 40, female 33. 13 patients were G 3a and 60 were G 3b. There were 43 genotype 1 patients, male 21, female 22. 8 patients were G 1a and 35 were G 1b. There were 62 genotype 6 patients, male 21 and female 41. 59 patients were G6 6L, 3 were G6 n and G6 m 1. Pre-treatment viral load ranges from 2502 to 48,300,000 IU/mL. IL28B gene polymorphism was not determined. All the patients achieved week 12 end of treatment response and week 12 Sustained Virological Response (SVR). It is to be concluded that week 12 SVR rate was 100 % in interferon eligible Myanmar patients with Chronic Hepatitis C infection treated with Sofosbuvir, Pegylated Interferon and Ribavirin triple drug combination for 12 weeks.

P-0083

The effect of peginterferon 2a and 2b in chronic HCV patients under response-guided therapy**Kuo-Chin Chang, Tsung-Hui Hu, Ming-Chao Tsai, Ming-Tsung Lin, Cheng-Kun Wu, Yi-Hao Yen**

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Background: Peginterferon (peg-IFN) is the standard therapy for chronic hepatitis C (HCV) infection. Studies comparing the efficacy of Peg-IFN2a/RBV and 2b/RBV in HCV-infected patients have shown conflicting results. We compared the efficacy for these agents in naive HCV patients underwent response-guide therapies in Taiwan. **Patients and methods:** 1111 chronic HCV patients were analyzed between 2009–2013 years. Efficacy outcomes were sustained virologic response (SVR), early virologic response (EVR) and rapid virologic response (RVR).

Results: Among 1111 patients, 508 (45.7 %) patients were treated with Peg-IFN-2a, and 603 (54.3 %) were treated with Peg-IFN-2b. The baseline characteristic was not significantly between two groups. After treatment, the RVR and EVR rates were 369 (72.5 %), 140 (27.5 %) for Peg-IFN-2a, and 413 (68.6 %), 189 (31.4 %) for Peg-IFN-2b. There was a higher response rate in every aspect of Peg-IFN-2b, but no significant difference between two groups. Male, HCV genotype non-1b, no liver cirrhosis, IL-28B 12979860 CC type, and HCV RNA $>6 \times 10^6$ IU/ml were easily to achieve RVR. In subset analysis, independent factors to predict SVR in RVR patients were HCV genotype non-1b, HCV viral load and non-LC. Furthermore, independent factors to predict SVR in EVR patients were HCV genotype 1b, non-LC, and IL28B 12979860 CC type. In LC patients, Peg-IFN-2b achieved high SVR rate than Peg-IFN-2a ($p = 0.005$)

Conclusion: In HCV-infected patients, the rates of SVR did not differ significantly between the two available Peg-IFN regimens. However, under response-guided therapies in Taiwan, the Peg-IFN-2b was more effective than Peg-IFN-2a therapy in HCV-infected patients with LC.

P-0084

The expression of circulating TFH cells and CD19 + B B cells in CHC patients with PEG-IFN therapy**Miao Zhang, Li Zhang, Hu Li, Zhiwei Chen, Aoran Luo, Bin Liu, Min Chen, Mingli Peng, Hong Ren, Peng Hu**

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Background: T follicular helper (TFH) cells are a subset of CD4 + T-helper cells and can activate B cells. This study aimed to investigate the role of circulating CXCR5 + CD4 + TFH cells, CD19 + B cells and the associated cytokines in patients with HCV infection.

Methods: The frequencies and phenotypes of circulating TFH cells and B cell subtypes were characterized using flow cytometry in chronic HCV patients treat with pegylated interferon (PEG-IFN α) plus ribavirin (RBV). The expression of IFN- γ , IL-12p70, IL-5, IL-13, IL-17F, IL-22, IL-23, TGF- β 1, IL-10 and IL-21 were analyzed.

Results: The frequency of CXCR5 + CD4 + T cells was significantly higher in patients with chronic HCV compared to HCs. Moreover, the expression of ICOS, PD-1, CD40L, and IL-21R in TFH cells also increased but failed to obviously change in B cell subtypes between patients with chronic HCV and HCs. Interestingly, chronic HCV patients with higher APRI or FIB-4 indexes have significantly lower percentages of memory B cells compared to patients with lower APRI or FIB4 indexes. The expressions of cytokines associated with the CD4 + Th lineage were higher in chronic HCV patients than in HCs, except for IL-21. Patients with rapid virological response (RVR) showed an increased CXCR5 + CD4 + T cell count and decreased PD-1 + CXCR5 + CD4 + T cell count compared to non-RVR patients at 4 weeks.

Conclusions: These data demonstrate that circulating TFH cells, B cells and CD4 + Th lineage-associated cytokines may play a role in HCV-related immune responses. Increased CXCR5 + CD4 + T cell counts may be related to virological response in chronic HCV infection.

P-0085

Success in difficult-to-treat patient with HCV-associated glomerulonephritis**Narina Sargsyants, Violeta Sargsyan, Hripsime Magdesieva, Naira Stepanyan**

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Introduction: Beyond the liver, chronic hepatitis C virus (HCV) infection leads to a multifaceted systemic disease. Viral elimination significantly reduced the rate of extra-hepatic deaths.

Case report: Patient A.H., female, 52 years old, HCV-infection was diagnosed in May 2011, because of systemic vasculitis with kidneys affection and nephrotic syndrome. Patient has been treated by rheumatologist and nephrologist: 6 courses of methylprednisolone and cyclophosphamide with short improvements. Monotherapy with PEG-IFN alfa-2a started in 04.04.2012.

Diagnosis: chronic hepatitis C (genotype 3, high viral load), HCV-associated systemic vasculitis with peripheral neuropathy, glomerulonephritis, nephrotic syndrome, ESRD, symptomatic hypertension, anemia, esophageal varices grade 2, depression. Interferonotherapy was started with simultaneously administration of methylprednisolone, furosemide, antihypertensives, epoetin, paroxetine and hydroxyzine. On 17 week of treatment hospitalized due to reactivation of systemic vasculitis with rash, oliguria/anuria, ascites, pericarditis, gingivostomatitis. Several times has been hospitalized because of bronchopneumonitis, severe anemia, enteric infection without antiviral treatment interruption. Patient received 48 injections PEG-IFN alfa-2a (first 24 weeks-135 mcg, next 24–90 mcg) with EVR, ETR, and significant improvement of renal function and symptoms of vasculitis. On 20 week after end-of-treatment due to exacerbation of vasculitis was checked HCV-RNA PCR-result was positive (relapse). Retreatment already with PEG-IFN alfa-2a 180 mcg and RBV 800 mg (withdrawn due to anemia development) without corticosteroids, started in 17.07.13. Duration of second therapy 48 weeks, better tolerated, with RVR, SVR and improvement of vasculitis symptoms.

Conclusion: This difficult-to-treat case reinforces necessity of antiviral treatment with SVR for hepatic and extrahepatic manifestations improvement.

Table 1. Main characteristics of the patient with HCV-associated glomerulonephritis

Viral load (IU/ml, Abbott Real time PCR)	
Before first course of therapy	29,501,424 (7.47 log)
On 4 week of first course of therapy	801 (2.90 log)
Before second course of therapy	3,707,369 (6.57 Log)
CD19⁺ total B lymphocytes abs./μl (reference range 90-660) – % (6-25)	30 – 6%
CD19⁺ CD20⁺ B lymphocytes abs./μl – %	20 – 4%
Rheumatoid factor (mg/L)	
At the beginning	929.7
Last	53.4
Hemoglobin (g/dL)	
At the beginning	8.64
Last	14.2
Creatinin clearance CrCl (Cockcroft-Gault) (mL/min)	
At the beginning	40
Last	49
Creatinin (μmol/L)	
At the beginning	245.0
Last	121.7
UREA (mmol/L)	
At the beginning	17.7
Last	9.2
ESR (mm/h)	
At the beginning	92
Last	17
Proteinuria (mg/dL)	
At the beginning	300
Last	none
Haematuria (cells/mL)	
At the beginning	250
Last	none
Main adverse events during first monotherapy course with PEG-IFNα-2a	Fever Anemia Depression Weight loss Pruritus Gingivostomatitis Bronchopneumonitis
Main adverse events during second antiviral course with PEG-IFNα-2a and RBV	Anemia Pruritus
Adjuvant medications/support during first monotherapy course with PEG-IFNα-2a	Epoetin Erythrocytar mass Methylprednisolone Antihypertesives Furosemide Omeprazole Paroxetine Hydroxyzine Levofloxacin Ciprofloxacin
Adjuvant medications/support during second antiviral course with PEG-IFNα-2a and RBV	Epoetin Paroxetine Hydroxyzine

P-0086

Telaprevir combination therapy in patients infected with hepatitis C virus genotype 4Bilgehan Aygen¹, Orhan Yildiz¹, Serpil Taheri², Zeynep Ture¹

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Introduction: The sustained virological response (SVR) rate for genotype 4 (G4) HCV-infected patients after pegylated interferon (PegIFN) and ribavirin (RBV) treatment is approximately 60 %.

Aim: To investigate the efficacy and safety of telaprevir (TVR) in combination with PegIFN/RBV in patients infected with G4 HCV who were previously treated with PegIFN/RBV and failed to achieve SVR.

Methods: The study included 10 patients: two prior relapsers, two prior partial responders, and six prior null responders to PegIFN/RBV treatment. The patients were given TVR/PegIFN/RBV for 12 weeks, followed by a 12–36 week PegIFN/RBV treatment. Rapid virological response (RVR), early virological response (EVR), extended rapid virological response (eRVR), SVR, and side effects of therapy were evaluated.

Results: The mean age of the patients was 48.90 years and seven were female. Interleukin (IL) 28B genotype was found CT in seven patients and two patients had cirrhosis. Treatment was stopped within

4 weeks because of the side effects in two patients. In three of the remaining eight patients (37.5 %) achieved HVR, RVR, and eHVR. SVR was obtained in two of these patients, but one patient had relapsed. SVR rates were 25 % in all patients.

Conclusion: TVR combination therapy had limited antiviral activity in this patient population. Further investigation of TVR combination therapy in patients with HCV G4 infection is warranted.

P-0087

Telaprevir-based triple therapy will be beneficial for patients of high alpha-fetoprotein levelKoji Takayama^{1,2}, Norihiro Furusho^{1,2}, Kazuya Ura^{1,2}, Satoshi Hiramine^{1,2}, Fujiko Kaseida^{1,2}, Yoshifumi Kato^{1,2}, Yuuki Tanaka^{1,2}, Eiichi Ogawa¹, Masayuki Murata¹, Jun Hayashi³

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Aim: Telaprevir combined with pegylated-interferon α (PEG-IFN α) and ribavirin (RBV) has improved a sustained virological response (SVR) rate for chronic hepatitis C. A high serum alpha-fetoprotein (AFP) and alanine aminotransferase (ALT) level is known as predictive marker for the occurrence of hepatocellular carcinoma after interferon-based treatment. This study was done to investigate the impact of this triple therapy on the AFP and ALT level.

Methods: 210 patients with chronic hepatitis C genotype 1 of high viral load (baseline serum hepatitis C virus RNA >5.0 logIU/mL) were divided into two groups by type of treatment: triple therapy with telaprevir, PEG-IFN α , and RBV for 24 weeks (n = 88), or dual therapy with PEG-IFN α and RBV for 48 weeks (n = 122). The relationship between virological response and the change in the serum AFP and ALT level from baseline to 24 weeks after the end of treatment was examined.

Results: Among patients with a high-baseline AFP level (≥ 10 ng/mL), the decline of AFP was significantly higher in the triple than in the dual therapy group (15.9 vs 1.6 ng/mL, P = 0.037). In contrast, among patients with a high-baseline ALT level (≥ 40 U/L), no significant difference was seen in the decline of ALT level between two therapy groups (52.9 vs 44.3 U/L, P = 0.168).

Conclusion: Compared with dual therapy, telaprevir-based triple therapy effectively reduced the serum AFP level among patients with a high-baseline AFP level. Triple therapy might produce more benefit for high-AFP patients than high-ALT patients.

P-0088

Work productivity impairment in Asian patients with hepatitis c treated with different regimensZobair M. Younossi^{1,2}, Maria Stepanova³, Henry LY Chan⁴, Mei-Hsuan Lee⁵, Ming-Lung Yu⁶, Yock Young Dan⁷, Moon Seok Choi⁸, Linda Henry³

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Background: Treatment of chronic hepatitis C (CHC) infection with interferon (IFN) and ribavirin (RBV) has been associated with impairment of work productivity (WP) in Caucasian CHC patients. However, this impact in the Asian CHC patients has not been fully investigated. Our aim was to assess WP of Asian CHC patients enrolled in different clinical trials.

Methods: Work Productivity and Activity Index (WPAI) instrument was administered at baseline, during, and post-treatment in subjects enrolled in 12 multinational clinical trials of sofosbuvir (SOF)-based regimens. Changes in WPAI scores (total work productivity impairment, absenteeism, presenteeism) from patients' own baseline levels in patients who reported to be of Asian ancestry were calculated during and after treatment.

Results: Out of 4485 CHC patients enrolled in these clinical trials, 106 were Asian (55.7 % male, 69.8 % treatment-naïve, 17.0 % cirrhotic, 14 % treated with LDV/SOF, and 85 % treated with IFN- and/or RBV-containing SOF-based regimens). By the end of treatment, WPAI score dropped significantly (-19.9 ± 30.1 points on a 0–100 scale, $p = 0.03$) in patients receiving RBV-containing regimens. A significant decline was also noted in the presenteeism component of WPAI (-8.0 ± 10.0 , $p = 0.02$). In contrast, WPAI scores did not change significantly in those who received LDV/SOF ($+1.79 \pm 6.24$, $p = 0.50$). Multivariate analysis showed that the end of treatment Work Productivity impairment was independently predicted by the use of RBV in the regimen (beta: -29.3 ± 12.7 , $p = 0.03$).

Conclusion: Interferon- and ribavirin-free LDV/SOF is superior to RBV-containing regimens in terms of patients' work productivity in Asian patients with chronic hepatitis C.

P-0089

Antiviral effects of daclatasvir and asunaprevir therapy on the liver function in hepatitis C

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Background: The combination therapy of daclatasvir (NS5A polymerase inhibitor) and asunaprevir (NS3 protease inhibitor) against chronic hepatitis C (CHC) patients were approved in Japan on September 2014. In this time, we evaluated antiviral responses and the effect on liver function of this combination therapy.

Methods: A total of 108 Japanese CHC patients at Osaka Medical College were enrolled in this study. A 60 mg of daclatasvir once a day in combination with 200 mg of asunaprevir twice a day was started in 24 weeks schedule. Antiviral and serological responses were evaluated every 2 weeks. Data shown mean \pm SD.

Results: Mean age was 69 ± 8.8 years old and 52 patients were male. Fifty-two patients were unresponsive and 10 patients were intolerant to previous peginterferon and ribavirin therapy. The others were naïve to antiviral therapy. Baseline mean HCVRNA level were 6.0 ± 0.7 logcopy/ml and 34 compensated cirrhotic patients were contained. At 4 weeks, serum HCVRNA levels were disappeared in the 78.3 % of

patients and 93.6 % of patients achieved sustained viral response (SVR) at 12 weeks after end of treatment. Serum ALT levels were significantly decreased and reached to normal levels within 4 weeks except for 7 patients. Serum albumin levels were significantly increased at 12 and 16 weeks, and serum AFP levels were significantly decreased at all point 4 weeks after treatment. Sixteen patients were discontinued this therapy, however 12 patients were achieved SVR.

Conclusion: The daclatasvir and asunaprevir combination therapy is very effective for CHC patients.

P-0090

Daclatasvir and asunaprevir for chronic genotype 1 hepatitis C virus infection in Japan

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Direct Acting Antivirals (DAAs) drastically changed the strategy of treatment for HCV infection. We have evaluated the efficacy and safety of daclatasvir (DCV) and asunaprevir (ASV) for the treatment of patients with chronic genotype 1 hepatitis C virus infection in Japan. We have analyzed the efficiency of 301 cases who have started with DCV and ASV therapy for at least 4 weeks. The patients include 114 men and 148 women, 31–87 in age and 174 chronic hepatitis and 86 hepatic cirrhosis. Initial data are as follows. Alb: 4.0 (2.5–5.1)g/dl, T-Bil: 0.7 (0.2–2.2)mg/dl, AST: 44 (10–175)IU/L, ALT: 39 (10–221)IU/L, GTP: 28 (10–139)IU/L, eGFR, 70.9 (24.0–177.2)ml/min, WBC: 4300 (1610–14,400)/ μ L, Hb: 13.2 (8.7–17.8)g/dl, Plt: 12.8 (2.5–40.9) $\times 10,000/\mu$ L, AFP: 5.9 (1.0–172.7)ng/ml, HCV RNA: 6.2 (3.3–7.3)LogIU/ml. In all patients, VR at 4 weeks rates was 86.3 %, VR at 12 weeks rate is 92.9 % and VR at 24 weeks 88.1 %. In 91 patients at age of over 75, VR at 4 weeks rates was 87.9 %, VR at 12 weeks rate is 93.2 % and VR at 24 weeks 92.1 %. DCV and ASV therapy was generally well tolerated. No patients experienced serious adverse events. But some patient discontinued treatment because of liver dysfunction and minor symptoms. We have obtained the satisfactory results with DCV and ASV therapy even in patients with aging, thrombocytopenia and renal dysfunction.

P-0091

ABT-493 and ABT-530 pharmacokinetics are similar in healthy Japanese, Chinese and Caucasian adults

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Aim: A next generation direct acting antiviral combination of ABT-493 (NS3/4A protease inhibitor discovered by AbbVie and Enanta) and ABT-530 (NS5A inhibitor) is being developed for the treatment

of chronic hepatitis C (HCV) genotype 1–6 infection. The objective of this study was to assess pharmacokinetics (PK) and safety of therapeutic doses of ABT-493 and ABT-530 administered to healthy Han Chinese, Japanese, and Caucasian subjects.

Methods: This Phase I, single center, multiple-dose, open-label study evaluated three doses of ABT-493 (100, 200 and 300 mg) and two doses of ABT-530 (80 and 120 mg) alone and in combination in 5 cohorts. Each cohort consisted of 9 Japanese, 9 Han Chinese and 9 Caucasian subjects (Table 1). Intensive PK assessments were performed on Days 1, 7, 8 and 14. PK parameters were estimated by noncompartmental analyses. Safety [adverse events (AEs), clinical labs, vital signs, ECG] was assessed throughout the study

Results: Following ABT-493 100, 200 or 300 mg QD and ABT-530 80 or 120 mg QD alone or co-administration, steady-state ABT-493 and ABT-530 exposures in Japanese and Han Chinese were comparable to Caucasians across dose levels on Day 14 (Figure 1). The nonlinear dose-exposure relationships for ABT-493 were similar across Japanese, Han Chinese and Caucasian subjects. No serious AEs were reported and no subject discontinued due to AEs. All AEs were mild or moderate in severity.

Conclusions: Ethnicity has no meaningful impact on exposures, safety or tolerability of ABT-493 or ABT-530 alone, or ABT-493 + ABT-530 combination

Figure 1. ABT-493 and ABT-530 Geometric Mean Exposures in Japanese, Han Chinese, and Caucasians at Different Combination Doses

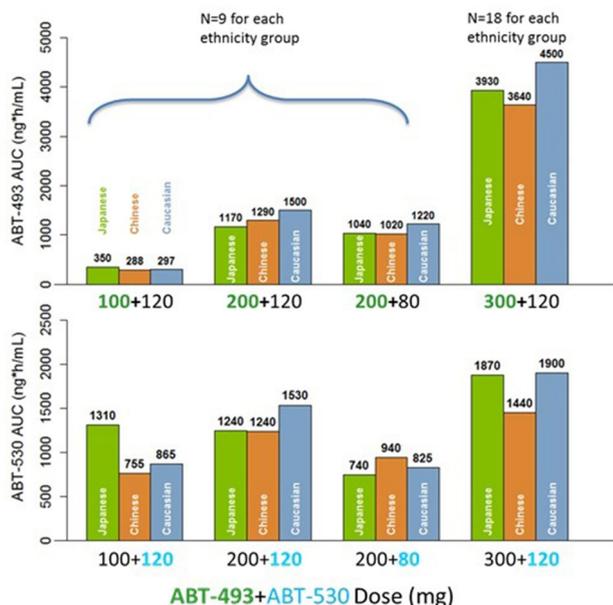


Table 1. Dosing Schematic

Cohort ^a	Number of Subjects ^b	Treatment	
		Day 1 to Day 7	Day 8 to Day 14
1	27	ABT-493 200 mg QD	ABT-493 200 mg QD + ABT-530 120 mg QD
2	27	ABT-493 300 mg QD	ABT-493 300 mg QD + ABT-530 120 mg QD
3	27	ABT-530 120 mg QD	ABT-493 300 mg QD + ABT-530 120 mg QD
4	27	ABT-530 80 mg QD	ABT-493 200 mg QD + ABT-530 80 mg QD
5	27	ABT-493 100 mg QD	ABT-493 100 mg QD + ABT-530 120 mg QD

a. Subject may only participate in one arm and one cohort.
b. N=9 each for Japanese, Han Chinese and Caucasians

P-0092

Daclatasvir and Asunaprevir improve the baseline parameters in chronic hepatitis C patients

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Background: The aim of this study was to investigate the efficacy of daclatasvir (DCV) and asunaprevir (ASV) combination therapy in chronic hepatitis C patients.

Methods: From September 2014 to June 2015, a total of 94 patients with chronic hepatitis C genotype 1b, received DCV 60 mg once daily and ASV 100 mg twice daily for 24 weeks in our institute. We investigate the clinical parameters including complete blood, HCV RNA, AFP level, and biochemical data prior the treatment and at every month were obtained prior to treatment, and every month during and after the treatment.

Results: Of all 92 patients 77 patients (83.9 %) had the prevalence of pre-existing protease inhibitor resistance associated amino acid variants (RAVs). 84 patients (84 %) completed treatment and 5 patients developed a virological breakthrough and 1 patient relapse. treatment, 95 % (80/84) patients achieved SVR4 and 93 % (65/70) achieved SVR 12, respectively. Each parameters changed from baseline to follow-up week 12 were below. Bilirubin, 1.1 mg/dl ± 2.2 to 0.86 mg/dl ± 0.87 (P = 0.158), AST 50.3 ± 38.8 to 27.1 IU/ml ± 13.1 (P < 0.001), ALT 46.7 U/ml ± 52.1 to 20.8 IU/ml ± 18.2 (P < 0.001), cholesterol 162.4 mg/dl ± 31.4 to 185.4 mg/dl ± 40.4 (P = 0.813), total protein 7.36 g/dl ± 0.64 to 7.32 g/dl ± 0.61 (P = 1.147), albumin 3.92 mg/dl ± 0.52 to 4.05 mg/dl ± 0.51 (P = 0.056), Cr 0.91 mg/dl ± 0.21 to 0.81 mg/dl ± 0.21 (P < 0.001), AFP 18.9 ng/ml ± 38.2 to 4.31 ng/ml ± 2.30 (P = 0.0026), WBC 4808/μl ± 1.75 to 5216/μl ± 1.62 (P = 0.007), hemoglobin 12.9 g/dl ± 1.52 to 12.6 g/dl ± 1.71 (0.113), Platelet count 14.0 × 10⁴/l ± 84.7 to 14.6 × 10⁴/μl ± 84.4 (P = 0.367), respectively. Mean level of AFP, AST, ALT, Cr, WBC at 12 weeks after treatment had decreased significantly from baseline.

Conclusion: This study showed that DCV and ASV, which interferon-free treatment of hepatitis C patients had not only well therapeutic effects but also improve each clinical parameters.

P-0093

Daclatasvir plus asunaprevir for hepatitis C infected patients in Shonai area Yamagata prefecture

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Background: Chronic hepatitis C virus (HCV) infection is a global health problem, but combinations of daclatasvir + asunaprevir (DCV +ASV) regimen improve outcomes for patients with hepatitis C virus (HCV) infection. We investigated the efficacy and safety of DCV +ASV regimen in patients infected HCV genotype 1b in Shonai area Yamagata prefecture.

Method: The subjects were 16 patients with HCV infection who received DCV +ASV for 24 weeks at Nihonkai General Hospital between December 2014 and July 2015. Serum alanine aminotransferase (ALT), serum HCV RNA level, alpha fetoprotein, and ferritin were measured. The primary endpoint was sustained virological response 12 weeks after treatment (SVR12).

Result: Sixteen patients received DCV +ASV treatment, and 75 % of patients had received pegylated interferon and ribavirin combination therapy [6 men and 11 women, aged from 41 to 77 years (age: 64.9 ± 9.4 years, mean \pm standard deviation (SD))]. Mean baseline viral load was 5.9 log IU/mL (range 4.6–6.5 log IU/mL). Pretreatment Y93H and L31M were detected in 4 and 1 of the 14 patients with available baseline NS5A sequences. SVR 12 was achieved by 87.5 % of all patients, 91.7 % of interferon-naïve patients and 75 % of interferon-treated patients, respectively. Two patients had virological breakthrough. Serum HCV RNA levels, ALT, alpha fetoprotein and ferritin were improved rapidly during treatment.

Conclusion: All-oral treatment with DCV + ASV for 24 weeks is well tolerated and achieve a high rate of SVR12 in patients with HCV genotype 1b infected patients who currently have no effective prior interferon-based therapy.

P-0094

Daclatasvir plus asunaprevir in difficult to treat HCV Gt1b population, EAP Turkey experience

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Introduction: Turkey has a 0.9–1.0 % prevalence of HCV infection with 91 % GT1b predominance. Daclatasvir (DCV) is a pangenotypic HCV NS5A inhibitor and Asunaprevir (ASV) is a HCV NS3 protease inhibitor approved for the treatment of chronic HCV infection.

Patients and methods: Sixty two patients were enrolled in the EAP. Ten of them (17.3 %) were naïve and the others (82.7 %) were treatment experienced. Treatment experienced patients were; null responders (17/48, 35.4 %), relapsers (18/48, 37.5 %) and IFN ineligible, intolerant 13/48, %27.1 respectively. Sixty-two percent (35/56) of patients were cirrhotic (Child Pugh A) and 35/58 (%60.3) had high viral RNA load (>800.000 IU/ml). All patients started DCV 60 mg/day and ASV 100 mg BID treatment for 24 weeks and 59 patients completed treatment.

Results: Overall SVR12 rate were 92.5 % (37/40). Cirrhotic patients 12/13 and non cirrhotic patients 24/26 had an SVR12 of (92.3 %). All

of naïve patients had SVR12 (%100) whereas treatment experienced patients had an SVR of 29/32 (%90.6). PegIFN/Ribavirin experienced group had an SVR12 of 92.3 % (24/26). All of PegIFN/Ribavirin/Protease Inhibitors experienced patients had SVR12 of %100 (6/6). The most common adverse events (25.8 % of patients) were fatigue, headache, mouth dryness and insomnia. There was no withdrawal from therapy due to adverse events.

Conclusion: 24 weeks of DCV + ASV is found to be highly effective, well-tolerated, interferon ribavirin-free regimen in this difficult to treat population of patients with prior pegIFN/RBV and pegIFN/RBV/PI failure in both cirrhotics and non cirrhotics.

P-0095

Daclatasvir/asunaprevir treatment reduces liver fibrosis

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Background: Daclatasvir (DCV)/Asunaprevir (ASV) treatment achieve extremely high SVR rate in chronic HCV genotype 1b infection. We examined the efficacy and side effects of DCV/ASV.

Patients and methods: 107 patients with chronic HCV infection received DCV/ASV from September, 2014 to September, 2015 in our hospital.

Results: HCV RNA became negative in more than 80 % after 4 weeks of treatment. 10 patients discontinued the treatment. The reasons were ALT elevation in 3, appetite loss in 2, breakthrough in 2, skin eruption in 1, hepatic encephalopathy in 1, and hepatocellular carcinoma in 1. AFP levels after 3 months treatment was significantly lower than the baseline. Hyaluronic acid (HA) levels at the end of treatment was significantly lower than baseline. Liver stiffness measured by fibroscan at the end of treatment was lower than baseline. Velocity of shear wave (Vs) measured by acoustic radiation force impulse (ARFI) did not significantly differ among the start, 3 months later, and the end of treatment. But, in the patients with baseline Vs of 1.7 m/s and higher, Vs at the end of treatment was significantly lower than baseline.

Conclusion: DCV/ASV treatment was effective to make HCV RNA negative. AFP levels and HA levels significantly decreased by the treatment. Liver stiffness by fibroscan significantly decreased by the treatment, and Vs also decreased in the patients with high baseline Vs. It is suggested that DCV/ASV treatment improve liver fibrosis.

P-0096

Efficacy and safety of asunaprevir and daclatasvir therapy for chronic hepatitis C patients

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Background and aims: We investigated the efficacy and safety of asunaprevir (ASV) and daclatasvir (DCV) therapy in Japanese patients with hepatitis C virus (HCV) genotype 1b infection.

Methods: In the Ehime Kan-en network, 217 patients with HCV genotype 1b infection (49.3 % treatment-naïve patients, 8.3 % relapsers, and 34.1 % non-responders) received ASV and DCV for 24 weeks. We assessed the safety of the treatment and the achievement of sustained viral response 4 weeks after treatment (SVR4).

Results: Of 217 patients, 50.2 % were men, and median age was 69 (40–86) years. The presence of resistance-associated NS3 and NS5A variants (RAVs) of HCV was evaluated in 98.6 % of patients; NS5A Y93, NS5A L31, and NS3 D168 RAVs were detected in 1.4, 0.9, and 3.2 % of the patients, respectively. Overall, SVR4 was achieved in 92.8 % patients (104/112), including 94.3 % treatment-naïve patients (50/53), 100 % (9/9) relapsers, and 90.0 % (45/50) non-responders. Treatment was discontinued because of severe adverse events in 22 patients (10.1 %), and causes included AST/ALT elevation (2.3 %), viral breakthrough (2.3 %), and prolonged prothrombin time (PT) (2.3 %). Discontinuation for PT prolongation occurred within 2–3 weeks after starting treatment.

Conclusions: ASV and DCV therapy for Japanese HCV genotype 1b patients achieved a high rate of SVR4. However, severe adverse events occurred in some cases. Careful monitoring of PT and AST/ALT may prevent severe adverse events.

P-0097

Efficacy of combination therapy of daclatasvir and asunaprevir for HCV genotype 1

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Introduction: In Japan, the combination therapy of daclatasvir (DCV) and asunaprevir (ASV) became available for the patients with hepatitis C virus C (HCV). The efficacy of this therapy showed better than the interferon-based therapy, although associations of resistance associated virus (RAV) have been shown with poor outcomes. Some patients without RAVs could not obtain sustained virological responses (SVR), of which the cause has not been clear.

Methods: Sixty-seven HCV-serogroup-1 patients with chronic hepatitis or liver cirrhosis received the combination therapy of DCV and ASV in our hospital between September 2014 and May 2015. The presence of RAV in non-structural (NS) regions of 3 and 5A was analyzed with direct sequencing. Genotypes were also done in regions of core and 5B. We investigated the factors associated with SVR.

Results: Of 26 patients including 6 patients who terminated the therapy for adverse events, SVR12 was achieved in 20 (76.9 %). Significant associations with SVR were observed for male patients but not for age, cirrhosis, or previous interferon therapy ($p = 0.02, 0.19, 0.35,$ and $0.40,$ respectively). Among the patients without SVR, RAV of 80K in NS3 was detected in two patients, other than RAV of 31M

and 93H in NS5A. Subgroup analysis in the patients without RAVs showed that male patients had better outcomes than female patients ($p = 0.03$).

Conclusion: The combination of DCV and ASV shows high antiviral efficacy in the patients with HCV serogroup 1. The RAV of 80K in NS3 is also associated with the therapeutic outcomes.

P-0098

Efficacy of daclatasvir plus asunaprevir for hepatitis C in patients non-positive for NS5A variants

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Aim: To reveal the effect of treatment with daclatasvir (DCV) plus asunaprevir (ASV) for hepatitis C patients non-positive for NS5A resistance-associated variants.

Methods: In total, 89 patients non-positive for the NS5A L31 or Y93 variant by PCR-Invader assay or direct sequence analysis were treated with DCV plus ASV. The virological response after 2 weeks and sustained virological response at week 12 (SVR12) following completion of treatment were examined. Seventy-four patients were divided into two groups according to their fold-over-zero (FOZ) values of the L31F, M, V and Y93 variants: the low FOZ value group (FOZ value less than the median) and high FOZ group (FOZ value greater than the median).

Results: Nine patients discontinued the therapy due to side effects; all were female ($p = 0.004$). The viral clearance rate at 2 weeks was associated with the FOZ value of Y93H, being 45.5 % (15/37) in the low FOZ value group and 19.4 % (7/37) in the high FOZ value group ($p = 0.043$). In the Y93H high FOZ value group, the viral clearance rate at 2 weeks was significantly lower than in the groups with high L31F, L31M or L31V FOZ values ($p = 0.018$). The SVR12 rate among patients who completed treatment was 97.8 % (45/46). A patient who experienced relapse after treatment showed the highest Y93H FOZ value.

Conclusion: Treatment of patients according to sex and Y93H FOZ value could result in a marked increase in the SVR rate.

P-0099

Evaluation of daclatasvir and asunaprevir exposures in non-Japanese Asians, Japanese, and Caucasians

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Background: The combination of daclatasvir (DCV; pangenotypic NS5A inhibitor) plus asunaprevir (ASV; NS3 protease inhibitor) has been generally safe and well tolerated in clinical studies and was the first all-oral regimen to be approved in Japan and Korea for treatment of HCV genotype 1b (GT1b) infection. This analysis evaluated the pharmacokinetics (PK) of DCV + ASV across different Asian populations and compared them with non-Asian data.

Methods: Data were pooled from several intensive PK sub-studies from selected DCV + ASV clinical trials in patients with HCV GT1b infection, including patients from mainland China (AI447-036, N = 19) and Japan (AI447-026, N = 40; AI447-031, N = 135), and a global study (AI447-028, 21 Asians [predominantly Taiwanese (81 %) and Korean], 13 non-Asians [92 % Caucasian]). DCV and ASV PK parameters were compared across ethnicities within these studies.

Results: Steady-state PK data by ethnicity are shown in the table. DCV exposures were generally similar across ethnicities. ASV exposure in mainland Chinese patients was similar to other non-Japanese Asian (predominantly Taiwanese) and Japanese patients, and higher than in Caucasian patients; these data were consistent with prior comparisons between Japanese and Caucasian patients. DCV + ASV safety and efficacy were similar between Asian and non-Asian patients.

Conclusions: DCV exposure was not affected by ethnicity. ASV exposures in non-Japanese Asian and Japanese patients are similar and higher than those observed in Caucasian patients. Efficacy and safety observations across studies/populations and prior exposure-safety/efficacy analyses suggest that these differences in ASV exposure do not translate into clinically meaningful differences in safety profiles.

	DCV			ASV		
	C _{max} ^a	AUC _{tau} ^b	C _{min} ^a	C _{max} ^a	AUC _{tau} ^b	C _{min} ^a
AI447-028 (Non-Asian)	981 (45)	9332 (41)	150 (49)	410 (142)	1329 (148)	38 (126)
AI447-028 (Asian)	1269 (54)	11665 (54)	198 (86)	703 (80)	2345 (82)	55 (156)
AI447-036 (China)	1155 (30)	12175 (37)	221 (54)	642 (64)	1910 (76)	45 (83)
AI447-026 (Japan)	1115 (37)	11878 (55)	253 (90)	647 (95)	2155 (80)	31 (90)
AI447-031 (Japan)	–	–	226 (68)	–	–	33 (103)

^a ng/mL; ^b ng•h/mL. All values are geometric means (%CV)

P-0100

Elbasvir/grazoprevir in cirrhotic patients with HCV infection

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Background: Treatment of cirrhotic HCV-infected patients (pts) often requires ribavirin (RBV) and long durations of therapy. An analysis of the efficacy/safety of elbasvir (EBR)/grazoprevir (GZR) ± RBV among compensated cirrhotics in the phase 2/3 EBR/GZR program was conducted.

Methods: Treatment-naïve (TN) and treatment-experienced (TE) pts with compensated cirrhosis received EBR/GZR ± RBV for 12 (TN)

or 12–18 weeks (TE) in 6 studies. The primary endpoint was HCV RNA < lower limit of quantification 12 weeks after end of treatment (SVR12).

Results: 169 TN and 233 TE pts with compensated cirrhosis were enrolled (male, 63 %; mean age, 56 years; HIV coinfected, 10 %; G1a, 54 %; G1b, 39 %; G4, 6 %; G6, 1 %; platelets <100,000/mm³, 27 %). Among TE pts, 51 % had prior null response to peginterferon + RBV and 15 % had failed therapy with a protease inhibitor + PR. SVR12 rates are presented in the table. Across TN and TE pts treated with EBR/GZR (no RBV), 78.1, 0.2, and 1.3 % reported an adverse event (AE), a drug-related SAE, or discontinuation due to an AE, respectively (vs 75.4, 0.2, and 0.8 %, respectively in noncirrhotics). Grade 3 hemoglobin decreases were 4 and 0 % in pts treated with and without RBV (no pt had a grade 4 decrease). On-treatment ALT >5 × ULN occurred in <1 % of pts.

Conclusions: In phase 2/3 studies, EBR/GZR for 12 weeks resulted in high efficacy among cirrhotic TN and GT1b-infected TE pts. For GT1a/4/6 TE pts, a 16/18-week regimen ± RBV, was highly effective. EBR/GZR regimens were generally well tolerated among cirrhotic pts.

	EBR/GZR Treatment Regimen	SVR12,* % (n/N)
Treatment-naïve*		
All	12 wks/no RBV	98 (135/138)
All	12 wks/+ RBV	90 (28/31)
GT1a	12 wks/no RBV	96 (73/76)
GT1b	12 wks/no RBV	100 (54/54)
GT4	12 wks/no RBV	100 (6/6)
Treatment-experienced		
All	12 wks/no RBV	89 (48/54)
All	12 wks/+ RBV	91 (74/81)
All	16/18 wks/no RBV	94 (46/49)
All	16/18 wks/+ RBV	100 (49/49)
GT1a	12 wks/no RBV	89 (31/35)
GT1a	12 wks/+ RBV	88 (29/33)
GT1a	16/18 wks/no RBV	96 (24/25)
GT1a	16/18 wks/+ RBV	100 (30/30)
GT1b	12 weeks/no RBV	100 (13/13)
GT4 [†]	All regimens combined	76.4 (13/17)
GT6 [‡]	All regimens combined	66.7 (2/3)

*No TN cirrhotic GT6 pts were included in this analysis.
[†]Includes 13 pts treated for 12 wks and 4 pts treated for 16/18 wks.
[‡]3 TE cirrhotic GT6 pts received 16/18 wks (± RBV) of therapy.

P-0101

Virological breakthrough and hepatic toxicity in the treatment with daclatasvir plus asunaprevir

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Background: Resistant associated variant (RAV) and hepatic toxicity are the concerned issues of daclatasvir plus asunaprevir for the treatment of chronic hepatitis C (CHC) genotype 1b (GT 1b). This study is to investigate the incidence of virological breakthrough and the hepatic toxicity in the real world.

Methods: Twenty-one patients of CHC, GT 1b had no RAV and received a 24-weeks, twice-daily, fixed-dose daclatasvir (30 mg) plus asunaprevir (200 mg). They had been treated for greater than 12 weeks. Virological breakthrough is defined as a confirmed increase in HCV-RNA of 1 log₁₀ IU/mL or greater from nadir or confirmed increase in HCV-RNA to greater than or equal to the assay lower limit of quantitation (LLOQ; 15 IU/mL) after a previous decline to less than the LLOQ. Hepatic toxicity is defined as ALT > 10X ULN or ALT > 5X ULN plus bilirubine >2mh/dl. Statistics were assessed by SPSS, version 16.0.

Results: The 21 patients were 62.5 ± 9.3 years, and included 9 males (42.8 %), 13 experienced (61.9 %), 14 cirrhotics (66.7 %). The baseline viral load (log₁₀) was 5.94 ± 0.83 IU/ml. Two (9.5 %) of them had virological breakthrough at week 4 and week 5. Both were experienced, one male and one female. The other 19 achieved RNA undetectable by week 4 and maintained till the end of treatment. Their AST/ALT were within normal limit in 14 (66.7 %) and within 2X of UNL in 5 (23.8 %), within 3X ULN in 1 (4.7 %).

Conclusions: In our study, virological breakthrough occurred in 9.5 % and early of treatment. No hepatic toxicity was noted.

P-0102

High SVR with DCV + ASV in HCV GT-1b Chinese, Koreans & Taiwanese without baseline NS5A polymorphisms

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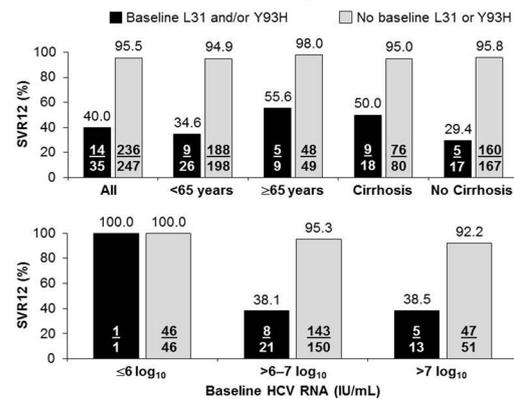
Background: Daclatasvir (DCV) plus asunaprevir (ASV) has demonstrated high sustained virologic response (SVR) in HCV genotype (GT-)1b infection. NS5A-Y93H and NS5A-L31 resistance-associated polymorphisms (RAPs) to DCV are known to impact DCV + ASV response in GT-1b-infected Japanese. The effect of RAPs on SVR at posttreatment week 12 (SVR₁₂) to DCV + ASV was explored in mainland Chinese, Koreans, and Taiwanese.

Methods: Pooled data from 2 studies of DCV (60 mg daily) + ASV (100 mg capsule, twice-daily) for 24 weeks in GT-1b-infected interferon/ribavirin-naïve and -experienced patients from mainland China, Korea, and Taiwan. Similar Japanese data (4 studies; n = 445) were pooled for comparison. SVR₁₂ with versus without baseline Y93H and/or L31 RAPs was compared by age (<65 vs. >65 years), cirrhosis status, and baseline HCV-RNA.

Results: SVR₁₂ and baseline NS5A sequences were available for 282 patients (126 mainland Chinese [45 %], 80 Koreans [28 %], 76 Taiwanese [27 %]). NS5A-Y93H and/or -L31 RAPs were observed pretreatment in 8 % mainland Chinese, 14 % Korean, and 18 % Taiwanese patients, compared with 19 % in Japanese. SVR₁₂ in all non-Japanese patients is shown (Figure); rates were broadly similar between countries and with Japanese data (Japanese: 96 % overall without RAPs, 41 % with RAPs). Responses were lower among patients with baseline RAPs. By contrast, SVR₁₂ in patients without RAPs was high (92–100 %), irrespective of cirrhosis, age, or baseline HCV-RNA.

Conclusion: At least 95 % of GT-1b-infected patients from mainland China, Korea or Taiwan without baseline NS5A-Y93H or -L31 polymorphisms who had HCV-RNA <7 log₁₀ IU/mL achieved SVR₁₂ on DCV + ASV, regardless of cirrhosis status and age.

Chinese, Korean and Taiwanese patients receiving DCV+ASV (studies AI447028, AI447036)



Nonvirologic failures censored (SVR4 with no subsequent follow-up, or discontinuation for reasons other than lack of efficacy within <28 days treatment). SVR₁₂ defined as HCV RNA < LLOQ with or without target detected at post-treatment Week 12. Missing Week 12 data imputed using the next available measurement. Success at Week 12 followed by relapse at next visit imputed as failure.

P-0103

Hemoglobin decrease with iron deficiency induced by DCV/ASV therapy for chronic hepatitis C

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Background: Hemoglobin (Hb) decrease has been supposed to be a relatively rare side effect of a combination therapy against hepatitis C virus, composed of the NS5A inhibitor daclatasvir (DCV) and the NS3/4A protease inhibitor asunaprevir (ASV).

Methods: The study was conducted on 75 patients with genotype 1b chronic hepatitis C who started combination therapy using DCV and ASV at St. Marianna University School of Medicine Hospital between September 2014 and December 2014.

Results: Among the 75 patients examined, the decrease in Hb by 1.5 g/dl or more in comparison with the values found at the initiation of treatment was observed in 11 individuals. The median (range) of the period until the achievement of a 15 g/l or more decrease in Hb levels was 8 (2–14) weeks. The mean value of the MCV at baseline and that at the time of the decrease in Hb levels were 91.3 and 87.9 fl, respectively. The mean serum iron levels at baseline and that at the time of the decrease in Hb levels were 70.6 and 49.8 mcg/dl, respectively. The median serum ferritin levels at baseline and that at the time of the decrease in Hb levels were 27.1 and 16.6 ng/ml, respectively. All of these changes were statistically significant.

Conclusions: These findings suggest the mechanism of the phenomenon is caused by iron deficiency. The underlying mechanism as well as the clinical impacts will need to be further examined.

P-0104

Impact of RAV dominancy on treatment outcomes in HCV GT1B patients for daclatasvir and asunaprevir

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Aim: Treatment of hepatitis C virus (HCV) genotype 1b infection with daclatasvir (DCV) and asunaprevir (ASV) markedly reduces sustained virological responses (SVRs) in patients with pre-existing Y93H resistance-associated variants (RAVs) in the non-structural protein 5A (NS5A) region. The aim of this study was to elucidate the dominancy of naturally occurring RAVs in viral quasispecies on treatment outcomes in patients with HCV.

Methods: In total, 138 patients were prospectively selected from 152 patients treated with DCV and ASV, where evaluation of treatment outcomes at 12 weeks post-treatment was possible. Pre-treatment RAVs in the NS3 and NS5A regions were detected by qualitative polymerase chain reaction (PCR)-Invader assays, and the ratio of Y93H RAVs in viral quasispecies was measured by quantitative PCR-Invader assays.

Results: By performing quantitative PCR-Invader assays, the Y93H RAV was detected in 25 patients, but not in 104 patients. Among patients with the Y93H RAV, the Y93H ratio was 1–25 % in 5 patients, 26–75 % in 7 patients, and ≤ 76 % in 13 patients. Overall, SVR at 12 weeks after the completion of treatment (SVR12) was 90.6 % (125/138), and those with Y93H ratios of < 1 %, 1–25 %, 26–75 %, and ≤ 76 % were 99, 100, 71, and 23 %, respectively. Thus, the SVR12 decreased as the HCV Y93H ratio increased ($p < 0.0001$).

Conclusion: The dominancy of pre-treatment RAVs of DCV and ASV affected its treatment outcomes, suggesting the importance of identifying dominant HCV RAVs against each direct-acting antiviral agents.

P-0105

Predictive markers of viral response to daclatasvir/asunaprevir in HCV genotype 1 patients

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Background and aims: Dual oral therapy with daclatasvir and asunaprevir leads to a high sustained virological response (SVR) rate

in patients infected with HCV genotype 1. However, this therapy has some side effects. Therefore, the prediction of SVR is very important. **Patients and methods:** Criteria for patient inclusion were chronic hepatitis and compensated cirrhosis with HCV genotype 1b. Treatment was started with daclatasvir (60 mg/day) and asunaprevir (200 mg/day) that continued for 24 weeks. A total of 648 patients were retrospectively enrolled. HCV RNA levels were measured before and 2, 4, and 12 weeks after the start of therapy by real-time PCR. L31 and Y93 resistant variants of the NS5A region were measured by direct sequencing and cyclecleave PCR methods. The age, sex, with or without cirrhosis, ALT, platelet count, albumin, gamma-GTP, type IV collagen, prothrombin time and AFP before treatment, simeprevir therapy history were also analyzed as baseline factors.

Results: In multivariate analysis, no Y93 resistant variants and no simeprevir therapy history were significantly associated with the SVR. The rates of undetectable HCV RNA at 2, 4, and 12 weeks after the start of therapy were 22.9 % (139/605), 74.8 % (428/572), and 96.7 % (464/480). The patients with rapid viral response (RVR; undetectable HCV RNA at 4 weeks) also had higher SVR rate (93.7 %) than non RVR patients (81.9 %) ($P < 0.0003$).

Conclusion: No Y93 resistant variants and no simeprevir therapy history are important factors to predict the SVR before therapy and RVR is during therapy.

P-0106

The combination therapy of Daclatasvir and Asunaprevir for chronic HCV Genotype 1b Infection

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Purpose: Oral interferon-free combination therapy was started from September 2014 in Japan. It is reported that the daclatasvir and asunaprevir combination therapy is able to get the highly therapeutic effect beyond the combination therapy with protease inhibitor, peg-interferon alpha and ribavirin in patients with HCV genotype 1b. In this study, we evaluated to clarify the anti-viral effect and adverse event daclatasvir and asunaprevir combination therapy.

Method: A total of 170 patients were enrolled at 5 centers in Miyazaki prefecture, Japan from September 2014 to September 2015. Patients received daclatasvir 60 mg once-daily plus asunaprevir 100 mg twice-daily. Anti-viral effect including rapid virological response (RVR), sustained virological response (SVR) at week 12 after the end of therapy and treatment-related adverse effect were examined.

Result: Mean age of the patients was 71 years old, 59.6 % of patients were female, 34.9 % were compensated cirrhosis, and 13.0 % had a history of HCC treatment. RVR and VR12 were achieved at 85.2, 80.0 % respectively. As for the RVR, there was significant difference according to pre-treatment viral load. Treatment-related adverse events were occurred in 35.6 %, including fever, headache, nasopharyngitis, increase of ALT was occurred in 17 (11.6 %). Serious adverse events (Grade ≥ 3) of elevation of ALT was observed in 5 patients.

Conclusion: The combination therapy of daclatasvir and asunaprevir was associated with high rates of RVR, SVR, and was tolerable treatment for elderly patients infected with HCV genotype 1b.

P-0107

Successful treatment with daclatasvir/asunaprevir of a HCV patient after failure of PEGIFN and RBV

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Case: A 60 year-old woman was diagnosed with HCV infection 9 years ago. Her first liver biopsy was in 2006 and has revealed as HAI 3 fibrosis 1. We began her first antiviral treatment for 48 weeks of pegylated interferon (PEGIFN)-alpha and ribavirin (RBV) in 2006. At the end of the treatment HCVRNA was negative. But HCV infection relapsed and persisted for the entire follow-up period. In 2012 we tried again to treat with PEGIFN and RBV. But she could not tolerate this second treatment. In 2014 we got a second liver biopsy and resulted as HAI 10 fibrosis 4. In BMS early access program we begin third regimen with daclatasvir (1 × 60 mg) and asunaprevir (2Conclusion: The combination therapy of daclatasvir and asunaprevir was associated with high rates of RVR, SVR, and was tolerable treatment for elderly patients infected with HCV genotype 1b. 100 mg) for 6 months. The real-time PCR technique revealed an HCVRNA viral load of 580,000 IU/mL, genotype 1b, IL28B heterozygous CT, before treatment. At the first month of therapy HCVRNA was negative. She has sustained viral response (SVR) at 3 month after the end of the therapy. We couldn't try telaprevir/boceprevir option for this patient because of the need to use with PEGIFN. However, HCV was susceptible to the telaprevir in the drug resistance analysis against HCV NS3 inhibitors before the treatment. Population sequencing technique was used in the drug resistance analysis of serin protease region (codons 31–181) of HCV and was interpreted in The Genafor/Arevir-geno2pheno drug resistance tool (Center of Advanced European Studies and Research, Bonn, Germany, <http://coreceptor.bioinf.mpi-inf.mpg.de/>).

Conclusion: We have 95 % HCV genotype 1 in Turkey. Daclatasvir/asunaprevir combination seems very suitable for our patients.

P-0108

EQ-5D results in Asian patients with HCV G1b receiving DCV + ASV who are IFN intolerant/ineligible

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Aims: To assess the impact of 24 weeks of daclatasvir + asunaprevir therapy, on overall quality of life as measured by EuroQol (EQ-5D) in IFN (±RBV) ineligible/intolerant patients enrolled in a phase 3 study from mainland China, Korea and Taiwan (N = 159).

Methods: The EQ-5D questionnaire, which assesses overall quality of life, was completed by subjects at baseline, every 4 weeks on treatment and at post treatment weeks 4, 12 and 24. Descriptive statistics for the EQ-5D utility score (between 0 and max 1.0 best health) from five dimensions (mobility, self-care, usual activity, pain/discomfort, and anxiety/depression), and Visual Analogue Scale (VAS) score (with endpoints at 100 best and 0 worse health state) were performed. Utility and VAS scores were also summarized by SVR12 status, where higher scores indicate better quality of life.

Results: Overall, the mean EQ-5D score at baseline was 0.94 and 80.69 (Utility score and VAS score, respectively). At follow-up week 12, the mean change from baseline was +0.01 (SE = 0.01) and 7.40 (SE = 1.30) respectively. When summarized by SVR12 status, the mean change from baseline in EQ-5D utility score at follow-up week 12 increased for responders, mean: 0.02; (SE = 0.01, N = 143) and decreased for non-responders: -0.12 (SE = 0.082); however, the number of non-responders with follow-up week 12 EQ-5D scores was small (N = 13). The EQ-5D VAS score increased for both responders (mean change from baseline 7.66; SE = 1.36) and non-responders (mean change from baseline 4.54; SE = 4.45).

Conclusions: EQ-5D assessments indicated that patients maintained a consistent quality of life after 24 weeks of DCV + ASV treatment.

P-0109

Ultra-deep sequencing for detection of low abundant drug-resistant HCV in patients receiving DAAs

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Background: Development of novel direct acting agents (DAAs) has extremely improved sustained viral response (SVR) rate in anti-HCV therapy. Although emergence of resistance-associated variants (RAVs) is known to be associated with treatment failure, the dynamics of RAVs during DAA combination therapy have been unveiled. The current study is aimed to clarify whether patients harboring various abundance of RAV at baseline might show an expansion of drug-resistant HCV clones in response to daclatasvir (DCV) plus asunaprevir (ASV) dual therapy.

Patients and methods: Among a total of 270 patients treated with DCV/ASV in our study group, 25 patients failed to achieve SVR. Serum samples were collected before treatment and on HCV recurrence. RAVs were determined by conventional direct-sequencing and ultra-deep sequencing method.

Results: Non-SVR patients consisted of 8 males and 17 females with mean age of 68.3 years. In 12 of 25 (48.0 %) non-SVR patients, NS5A-RAVs were not detected by conventional direct-sequencing at baseline. However, ultra-deep sequencing detected various abundance of RAVs including NS5A-Y93H (median 3.47 %, range 0.72–32.6 % in average coverage of 43,680) in 8 patients. On relapse, DCV-resistant HCV variants with Y93H became the dominant population in 6 patients (median 98.2 %, range 50.0–99.8 %), and most of them acquired other major RAVs at NS5A-L31, Q54, or NS3-D168 during or after DCV/ASV administration.

Conclusion: Ultra-deep sequencing can detect quite a low level of preexisting drug-resistant HCV, which may be one of the significant causes for HCV recurrence in patients in whom direct sequencing failed to detect the RAVs prior to DAA treatment.

P-0110

Efficacy and safety of daclatasvir and asunaprevir for patients infected with HCV genotype 1

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Background and aims: Interferon-free treatment for hepatitis C genotype 1 by the NS5A inhibitor daclatasvir and NS3/4A protease inhibitor asunaprevir has been available since September, 2014 in Japan. The efficacy and safety of 60 mg daclatasvir and 200 mg asunaprevir for 24 weeks were evaluated.

Methods: 102 patients were treated: 66 (65 %) were chronic hepatitis and 36 (35 %) were compensated cirrhosis.

Results: 49 (48 %) were male, 37 (36 %) had been previously treated: 11 % with peginterferon alone, 20 % with peginterferon and ribavirin, 5 % with peginterferon and ribavirin and simeprevir. HCV-RNA became undetectable in 74 % at 4 weeks, 91 % at 12 weeks (Early virological response, EVR), 78 % at 24 weeks (End of treatment response, ETR). During the treatment, 8 patients had viral breakthrough. The resistant mutations were newly detected in 2 patients, and the number of mutation increased in 2 patients: 1 patient had D168V, another had Y93H before the treatment. In univariate analysis, ETR rates were significantly higher among patients who achieved EVR (94 %) than those who could not (0 %): $p < 0.001$, and lower among patients who had viral breakthrough (0 %) than those who had no it (97 %): $p < 0.001$, and lower among patients who had Y93H mutation (0 %) than those who had no it (83 %): $p < 0.01$. In multivariate analysis, the statistically significant predictor of ETR was viral breakthrough alone (odds ratio: 0.017, 95 % confidence interval: 4.4×10^{-4} –0.72, $p = 0.033$). 5 patients (4.9 %) discontinued the treatment because of adverse events: 1 of hepatic decompensation, 2 of fever and 2 of double vision. The most common adverse events were elevation of ALT (19.6 %), fever (3.9 %) and pruritus (3.9 %). No differences of AE rates were demonstrated between CH and LC ($p = 0.785$): among CH, AE rates were 21 % and among LC, those were 25 %.

Conclusion: The combination of daclatasvir and asunaprevir achieved high rates of virological response in patients infected with HCV genotype 1, including those with cirrhosis with mild and tolerable adverse events.

P-0111

High SVR rates with ABT-493 + ABT-530 in non-cirrhotic patients with HCV genotypes 1, 2, 3 infection

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Aim: Assess efficacy and safety of co-administered ABT-493, an NS3/4A protease inhibitor identified by AbbVie and Enanta, and ABT-530, an NS5A inhibitor, in Phase 2 studies with 12 week treatment duration in non-cirrhotic, HCV treatment-naïve or interferon-experienced patients with chronic HCV genotype (GT) 1, 2, or 3 infection.

Methods: Enrolled patients received once-daily ABT-493 + ABT-530, at varying doses, \pm weight-based ribavirin for 12 weeks. Efficacy and safety are reported.

Results: Seventy-nine patients with HCV GT1 enrolled in the SURVEYOR-I study (81 % had GT1a). In the SURVEYOR-II study, 74 patients with GT2 (82 % GT2b) and 121 patients with GT3 (98 % GT3a) were enrolled. Across treatment arms, sustained virologic response at post-treatment week 4 (SVR4) was achieved by 97–100 % of patients with GT1, 96–100 % with GT2, and 93–94 % with GT3. One GT1-infected patient experienced relapse by post-treatment week 4, no GT2-infected patients experienced virologic failure but one was lost to follow up, and 8 GT3-infected patients did not achieve SVR4 due to post-treatment relapse ($n = 3$), on-treatment breakthrough ($n = 2$), early discontinuation ($n = 2$), or missing data ($n = 1$). SVR12 data will be available for presentation. Adverse events (AEs) were mostly mild, with common AEs in >10 % of all patients being fatigue (20 %), nausea (16 %), and headache (15 %). Four serious AEs were reported, though none were deemed related to treatment.

Conclusions: ABT-493 + ABT-530 \pm ribavirin for 12 weeks provided high SVR4 rates and was well tolerated in patients with HCV GT1, 2, or 3 infection. Ribavirin did not enhance SVR in this study. This potent once-daily regimen is being further evaluated.

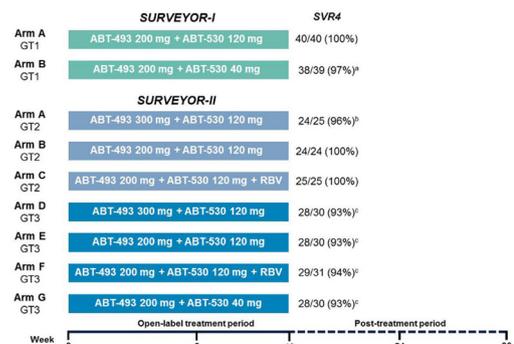


Figure legend: These phase 2, open-label studies consisted of a 12-week treatment period during which patients received ABT-493 + ABT-530 once daily, with or without ribavirin, followed by a 24-week post-treatment period. Presented are the SVR4 data for each treatment arm. SVR, sustained virologic response at post-treatment week 4 (SVR4); GT, genotype; RBV, ribavirin.

^a In Arm B of SURVEYOR-I, one patient with GT1 infection experienced relapse by post-treatment week 4.

^b In Arm A of SURVEYOR-II, one patient with GT2 infection discontinued study drugs early due to loss of follow up and did not achieve SVR4.

^c In SURVEYOR-II, 8 patients with GT3 infection did not achieve SVR4. In Arm D, one patient experienced relapse by post-treatment week 4 and another patient had missing data. In Arm E, 2 patients experienced relapse by post-treatment week 4. In Arm F (included RBV), one patient had on-treatment breakthrough and one patient discontinued treatment early due to AEs (abdominal pain and feeling hot). In Arm G, one patient had on-treatment breakthrough and one patient discontinued study drugs early due to loss of follow up.

P-0112

Indirect comparison of daclatasvir + asunaprevir vs sofosbuvir/ledipasvir for HCV in Japanese patients

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Aims: To compare daclatasvir and asunaprevir (DCV + ASV) versus sofosbuvir/ledipasvir (SOF/LDV) for HCV genotype 1 in Japanese patients without NS5A polymorphisms.

Methods: All published Phase 3 trials of DCV + ASV or SOF/LDV conducted in Japan were included. Individual patient data from two trials of DCV + ASV and published summary data from one trial of SOF/LDV were available. DCV + ASV-treated patients without baseline NS5A polymorphisms at L31F/I/M/V, Y93H, L28 M, and R30Q were included and subject to the enrollment criteria of the SOF/LDV trial. To adjust for cross-trial differences, DCV + ASV-treated patients were weighted to match reported summary baseline characteristics from the SOF/LDV trial. Sustained virologic response at week 12 post-treatment (SVR12) and discontinuation due to adverse events (AEs) were compared between the treatments.

Results: Of the 363 DCV + ASV-treated patients, 241 did not have NS5A polymorphisms at baseline. Of these, 216 met the inclusion criteria of the SOF/LDV trial (n = 171) and were included in the analysis. Before adjustment, the SVR12 rate was lower (96.3 % vs. 100 %; p = 0.011) and rate of discontinuation due to AEs was higher (3.7 % vs. 0.0 %; p = 0.011) among patients treated with DCV + ASV than SOF/LDV. After adjustment, the rate of SVR12 and discontinuation due to AEs was similar between DCV + ASV and SOF/LDV-treated patients (SVR12: 98.7 % vs. 100 %; p = 0.352; discontinuation: 1.3 % vs. 0.0 %; p = 0.321).

Conclusions: After adjustment for differences in baseline characteristics, DCV + ASV and SOF/LDV were associated with similar efficacy and discontinuation due to AEs in Japanese patients infected with HCV genotype 1 who did not have baseline NS5A polymorphisms at L31F/I/M/V, Y93H, L28 M, and R30Q.

P-0113

A prospective evaluation of a combination of sofosbuvir/ledipasvir performance in HCV infection

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Background and aims: We have enrolled 135 hepatitis C patients infected with genotype 1 and 4 in a clinical protocol of all oral exclusive treatment schedules recently accomplished in the Portuguese guidelines for hepatitis C treatment.

Methods: Genotype 1 and 4 HCV infected patients were treated with a fixed-dose combination comprising the nucleotide polymerase inhibitor sofosbuvir (SOF) 400 mg plus ledipasvir (LDV) 90 mg. They were stratified according to HCV genotype, fibrosis and prior

treatment response. Viremia was sequentially evaluated at baseline, week 4, 8, 12 and in the prolonged regimen, at week 24, all with follow-up blood sampling at week 12 post treatment.

Results: A total of 135 patients (mean age 57.1 ± 12.1) were evaluated: 58 % males, 73 % carried the non-CC IL 28B allele, 47 % genotype 1a infection and 41 % genotype 1b, 65 % were prior treatment non-responders/relapsers and 49 % had cirrhosis. Rapid virologic response (undetectable viremia at week 4) was achieved in 84 % of the patients and all (100 %) had undetectable viremia at week 8, 12 and 24. Treatment was well-tolerated and only one patient discontinued treatment, at week 11, due to symptomatic bradycardia (with concomitant use of amiodarone). No significant laboratory abnormalities were observed and ALT values normalized in 82 % of the patients at week 4.

Conclusions: In real life experience, SOF/LDV fixed dose combination tablet is highly effective, safe and well tolerated in treatment-naive and treatment-experienced, genotype 1 and 4 HCV-infected patients, achieving undetectable viremia in 100 % at week 8, so remaining till the end of treatment schedule.

P-0114

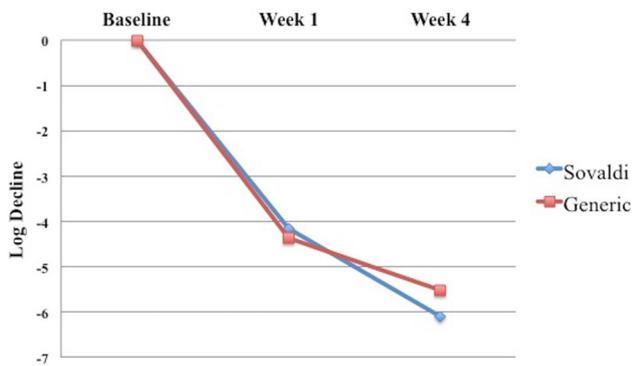
Comparative efficacy of generic sofosbuvir versus SOVALDI with ribavirin for hepatitis C

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Over 70 % of the HCV epidemic is present outside these regions with limited access to direct acting antiviral medications. Generic sofosbuvir is now available in India at less than 1/10 the cost of SOVALDI. We compare the early on-treatment antiviral efficacy and safety of generic sofosbuvir with SOVALDI. From December 2014 to August 2015, 39 patients were enrolled to receive 24 weeks of sofosbuvir and ribavirin at the Institute of Liver Diseases, Hepato Pancreato Biliary Surgery and Transplant, Global Hospitals, Mumbai, India. Thirty-six patients have week 4 on-treatment viral loads available. Twenty-one (58 %) received SOVALDI, and 14 (39 %) received generic sofosbuvir. The majority of patients were female (57 and 64 %) and mean age in both groups was 55 years. Safety profiles were collected as reported by patients or abnormalities in laboratory tests. Mean baseline hepatitis C viral load was 12329593 IU/ml in the SOVALDI group, and 4,808,333 IU/ml in the generic group (p = 0.26). There was difference in cirrhosis status at pre-treatment (p = 0.01), but not genotype (p = 0.12). Mean log decline in viral load from baseline to week 1 was -4.15 log among patients receiving Sovaldi, and -4.36 log among patients receiving generic sofosbuvir (p = 0.67). Log decline from baseline to week 4 was -6.1 log and -5.2 log respectively (p = 0.33).

Conclusion: Antiviral effectiveness of generic Sofosbuvir is comparable to SOLVADI in patients with all genotypes receiving HCV therapy. Bioequivalency of generic directly acting agents need to demonstrated before wider use of these agents are advocated.

On-Treatment Viral Load Decline

P-0115

Cost-effectiveness analysis of sofosbuvir for treatment-naïve G1 chronic hepatitis C in China

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Background: Several highly effective new direct-acting antiviral drugs with high sustained virologic response rate for the treatment of chronic hepatitis C (CHC) recently has made available in high-income countries. There was no evidence of their cost effectiveness in China.

Method: This study compared the clinical outcome and cost effectiveness of Sofosbuvir (SOF) based treatment with current best available treatment and no treatment in China, using a decision analytic Markov model. The study was conducted at payer's perspective. Direct medical cost was used. The target cohort was treatment-naïve genotype 1 CHC Chinese patient aged 55.

Results: With SOF drug price of RMB 520,000 per 12 week treatment, the average life time direct medical cost was RMB 591,167 for a CHC patient, with incremental cost effectiveness ratio (ICER) = RMB 184,685 /QALY compared to no treatment, and ICER = RMB 1.3 million /QALY compared to current treatment, both much higher than the willingness to pay level of China ($1 \times \text{GDP/capita}$). With SOF drug price of RMB 6000 per 12 week treatment, the average life time direct medical cost was RMB 77,166. SOF based treatment was cost saving compared with both current and no treatment. Deterministic and probabilistic sensitivity analysis confirmed the robustness of results.

Conclusions: For untreated genotype 1 CHC patients in China, SOF-based treatment can achieve better clinical outcome compared with current treatment regimen. However, the cost-effectiveness of SOF-based treatment depends heavily to the drug price. At US price, SOF-based treatment is not cost-effective in China. Negotiation to lower the price is strongly recommended.

P-0116

Cost-effectiveness analysis of sofosbuvir plus ledipasvir CHC GT1b:real life practice in China

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Background and aims: Pan-oral interferon (IFN)-free regimens is made available for chronic hepatitis C (CHC). We evaluate the cost-effectiveness of 12-week sofosbuvir plus ledipasvir (Harvoni®, Gilead) compared to IFN/RBV in treatment-naïve CHC GT1b patients in real life practice.

Methods: Real-life data was collected from a Hong Kong-Beijing Liver Centre. A Markov transition model was constructed based on the natural history of HCV infection by sex and cirrhosis stages. We estimated the quality-adjusted-life-years (QALYs), lifetime cost of HCV infection and incremental cost-effectiveness ratio (ICER). One-way and probabilistic sensitivity analysis and were undertaken to assess parameter uncertainty.

Results: The SOF/LDV regimen added 1.4 QALY compared to SOC, at an incremental cost effectiveness ratio (ICER) of US\$37,755 per additional QALY. The ICERs ranged from US\$57,106 to US\$27,729 with respect to sex and presence of cirrhosis. The ICERs were lower in patients with cirrhosis than those without cirrhosis. One-way sensitivity analysis showed that the results were most sensitive to utility scores after successful treatment, cost of SOF/LDV treatment, discount rate, and transition probability of decompensate cirrhosis to death status. At a willingness to pay threshold of US\$45,564 (6 times GDP per capita in China) per QALY, SOF/LDV regimen had a 55 % probability of being cost-effectiveness.

Conclusion: The IFN-free regimen is not cost-effective in most Chinese HCV genotype 1b patients mainly due to the high cost of IFN-free regimen and relatively low GDP per capital in China.

P-0117

Quality of Life of Japanese Patients with Chronic Hepatitis C Treated with Ledipasvir and Sofosbuvir

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Background: The Interferon (IFN)-free regimens for chronic hepatitis C (CHC) have high efficacy and superior health-related quality of life (HRQOL) in European/North American patients. The impact of

these regimens on HRQOL of the Japanese CHC patients is not known.

Methods: Short Form-36 was administered before, during and after treatment to CHC patients with genotype 1 treated with Ledipasvir/Sofosbuvir ± ribavirin (LDV/SOF ± RBV) for 12 weeks and genotype 2 treated with SOF + RBV for 12 weeks in clinical trials. HRQOL data was analyzed with reference to treatment regimens and clinical factors.

Results: 494 patients were included in this analysis (19 % cirrhotic, 69 % genotype 1, 52 % treatment-naïve; 153 received SOF + RBV, 170 received LDV/SOF + RBV, 171 received LDV/SOF). The SVR-12 rates for these regimens were 97, 98 and 100 %, respectively. Patients treated with LDV/SOF, SOF + RBV or LDV/SOF + RBV regimens had similar HRQOL scores at baseline. During treatment, more adverse events were experienced by those treated with RBV-containing regimens (46 vs. 22 %, $p < 0.0001$). The decrements in HRQOL were also significant in RBV group: up to -3.8 points (treatment week-4), -5.2 (treatment week-12), -3.2 (post-treatment week-12) (all $p < 0.001$). In contrast, RBV-free regimen (LDV/SOF) was associated with an improvement in HRQOL up to $+4.1$ throughout the treatment (all $p < 0.01$). In multivariate analysis, the use of RBV was independently associated with lower HRQOL during and after treatment (beta up to -6.4 points, $p = 0.0001$).

Conclusions: Japanese CHC patients treated with RBV-containing regimens show mild impairment of HRQOL. In contrast, patients treated with LDV/SOF not only showed high efficacy but also improvement of HRQOL.

P-0118

Characterizing resistance in patients receiving SOF or LDV/SOF in Korea, Japan, and Taiwan

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Background: In Korea, Japan and Taiwan, approximately 1–4 % of the population are infected with HCV. Sofosbuvir (SOF)-based regimens provide safe and highly effective treatment for patients infected with HCV genotype (GT) 1 or 2. The impact of preexisting resistant-associated variants (RAVs) on treatment outcome and emergence of RAVs at virologic failure in patients treated with SOF-based regimens was evaluated.

Methods: Across 4 Phase 3 studies, LDV/SOF ± RBV ($n = 519$) or SOF + RBV ($n = 369$) was administered for 12 weeks to Korean, Japanese and Taiwanese patients with HCV GT1 or GT2 infection, respectively. NS5A and/or NS5B deep sequencing (1 % cut-off) was performed on patient samples at baseline and virologic failure.

Results: Virologic failure rates were low; 0.6 % in GT1 and 1.9 % in GT2 patients. In GT1 patients treated with LDV/SOF ± RBV, 22 % (114/517) had HCV with baseline NS5A RAVs with 112/114 (98 %) achieving SVR12. Y93H and L31 M were the most commonly observed baseline NS5A RAVs. Twelve GT1 patients (2 %) had NS5B RAVs (no S282T) at baseline and all achieved SVR12. Three GT1 patients relapsed; two had NS5A RAVs at baseline and relapse,

and one had treatment emergent Y93H. Among GT2 patients treated with SOF + RBV, seven experienced virologic failure, and no baseline or treatment emergent NS5B RAVs were detected.

Conclusions: Low rates of virologic failure were observed and were not associated with SOF resistance variants. Baseline NS5A or NS5B RAVs did not affect the treatment outcome following 12 weeks of treatment with LDV/SOF ± RBV or SOF + RBV in patients with GT1 and GT2 infection, respectively.

P-0119

Detection of hepatitis C virus NS5A L31/Y93 mutations in patients with hepatitis C

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Background and aims: Interferon-free treatment for HCV infection was recently enabled by the combination of NS5A inhibitor and protease inhibitor in Japan, but mutations conferring resistance to NS5A inhibitor were observed. Here we focused on those at L31 and Y93 of NS5A reported in infection with genotype 1b HCV, and investigated the emergence in patients with genotype 1b HCV infection.

Methods: After isolation of viral RNA from patient serum and reverse transcription, the region encompassing the mutations was amplified by nested PCR. Subsequently, L31/Y93 mutations were examined by direct sequencing. Plasmids encoding NS5A fragments with L31F or Y93H were constructed.

Results: Reverse-transcribed viral RNA from serum of HCV-infected patient was used for the nested PCR with primers designed according to genome sequences of HCV genotype 1b. We next determined the best efficient and specific primer set in amplification and subsequent direct sequencing. The method was able to sense each mutation in the mixture of the plasmids encoding L31F or Y93H, with the individual mutation content of 30 % and 5 %, respectively. Out of 102 cases, L31F/M and Y93H were observed in 5 (5 %) and 12 (12 %), respectively, and both emerged in the two breakthrough cases without L31/Y93 mutations before the treatment with daclatasvir (NS5A inhibitor, BMS) and asnaprevir (protease inhibitor, BMS).

Conclusions: We newly generated a detection system of NS5A inhibitor resistance mutations in NS5A of HCV from patient serum. Our system could help to develop treatment strategies to evade the viral resistance and achieve SVR.

P-0120

Real-world effectiveness and cost/SVR: ledipasvir/sofosbuvir (LDV/SOF) for chronic HCV (CHC) treatment

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Background: With the emergence of highly effective, safe therapies for CHC and their anticipated demand, optimal resource allocation is

essential. The cost per sustained viral response (cost/SVR) provides insight into amount spent for therapy success. This study assessed safety, effectiveness, and cost/SVR associated with LDV/SOF therapy in German clinical practice.

Methods: The first CHC patients treated with LDV/SOF in a single centre were analyzed using descriptive statistics.

Results: 115 patients (51.3 % 8 weeks, 44.3 % 12 weeks, 4.4 % 24 weeks) initiated LDV/SOF treatment between 24/11/2014 and 03/03/2015. Mean age 52 years; 60 % males; 89.6 % had at least one comorbidity; 57 % GT1a, 32 % GT1b, 6 % GT3, 4 % GT4; 34 % F0, 19 % F1, 13 % F2, 11 % F3, 23 % F4. Median baseline HCV RNA was 0.97 million IU/ml. 6 % (2 %) of patients were HIV (HBV) co-infected. 21 % of patients (79 % F4) had ribavirin added. Among patients with available data, SVR4 was 99 % (92/93) and 100 % SVR12 (13/13). One treatment experienced F4 patient did not achieve SVR4. 6.9 % (5.2 % treatment-related) experienced grade 3/4 adverse events (AE), with no AE-related discontinuation. 1.0 % of costs were non-therapy. The median cost/SVR4 was Euro53,025. 73 % of naive and 66 % of non-cirrhotic (NC) patients were on 8 weeks duration; median cost/SVR4 was 59 and 58 % lower in NC (Euro46,775) and naive (Euro46,272) patients. SVR12 and cost/SVR12 will be available by November 2015.

Conclusion: With a good tolerability profile, monitoring and AE-related costs were minimal for LDV/SOF regimens. The cost/SVR is significantly lower in naive and NC patients treated for 8 weeks, indicating economic benefits from highly effective and well-tolerated first-line therapies and early treatment.

P-0121

Real-world effectiveness-8 weeks (8 weeks) ledipasvir/sofosbuvir (LDV/SOF) for chronic HCV (CHC) treatment

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Background: LDV/SOF single tablet regimen (STR) is approved in Europe for CHC patients with genotypes (GT) 1, 3 and 4. According to the summary of product characteristics (SmPC), 8w may be considered in treatment naive, non-cirrhotic patients with HCV RNA <6million IU/mL. Our aim was to characterize the population receiving 8w LDV/SOF and to describe clinical practice outcomes.

Results: 88 non-cirrhotic patients initiated 8w treatment with LDV/SOF (no ribavirin added) between 21/11/2014 to 07/04/2015. Median HCV RNA at baseline was 851,138 (Min-Max: 11–18,620,871) IU/ml, two F0 patients had HCV RNA ≥6 million (11,481,536 and 18,620,871) IU/mL. 99 % of the patients were treatment-naive (one relapser after previous INF/RBV therapy). Mean age 49.6 years; 44.5 % males; 53.4 % GT1a, 44.3 % GT1b, 2.3 % GT4; 50.0 % F0, 27.7 % F1, 18.2 % F2, 4.6 % F3. 4.6 % were HIV co-infected with no HBV co-infections. To date, no patient has discontinued therapy. Two patients experienced adverse events: one unrelated and one possibly related to treatment. Among patients for whom data is available, both SVR4 (80/80) and SVR12 was 100 % (72/72). Complete results will be available at the time of presentation.

Conclusion: 8w LDV/SOF is predominantly prescribed according to the SmPC. Preliminary results indicate that LDV/SOF is a highly effective, safe, well-tolerated treatment option with few adverse events or discontinuations reported.

P-0122

RVR response in HCV related compensated CLD with triple therapy containing sofosbuvir in Bangladesh

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Aim: Aim was to assess safety and efficacy of triple therapy containing world's first generic sofosbuvir and generic injection pegylated interferon and generic ribavirin in hepatitis C virus (HCV) related compensated chronic liver disease (CLD).

Methods: Sixteen patients were included. Informed, written consent was obtained from all. There were 9/16 (56.3 %) males and 7/16 (43.7 %) females. They were treated with tablet sofosbuvir (400 mg) (Tab. Hopenavir, Incepta Pharmaceuticals, Bangladesh) once daily in combination with generic injection pegylated interferon (180 µ) subcutaneously once weekly and generic capsule ribavirin (200 mg) 1000 mg daily in two divided doses for 12 weeks. Tab. Hopenavir used is the world's first generic sofosbuvir introduced in Bangladesh in February 2015.

Results: All had HCV genotype 3 compensated CLD. Ten completed 4 weeks treatment at data analysis and 4 completed 12 weeks. Nine had rapid virologic response (RVR) (HCV RNA <20 IU/ml). At baseline HCV RNA was 21,100–6,700,000 IU/ml (mean 29,094,676.8 IU/ml). Three of 4 completing 12 weeks treatment attained end of treatment response (ETR). Only patient failing to achieve RVR had HCV RNA reduced from 4,452,000 IU/ml to 44,258 IU/ml at 4 weeks and the patient not attaining ETR had RVR with HCV RNA declining from 181,905 IU/ml at baseline to 12,526 IU/ml at end of treatment. No significant adverse event was noted in any and no dose reduction was necessary.

Conclusion: Treatment with generic anti-virals including world's first generic sofosbuvir is safe and promising. Definite comment can be made once patients are evaluated at 12 weeks off treatment.

P-0123

Sofosbuvir and ledipasvir for SVR12 in sickle cell disease with HCV.SLASH C Trial

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Background: CHC is no longer a clinical challenge in the era of DAAs. CHC and SCD contributes added challenges (sickle cell hepatopathy, splenic dysfunction, accelerated fibrosis secondary to anemia, iron overload and LPS induced mitochondrial injury). Historical management with IFN and RBV causes fatal hemolysis, severe anemia and sepsis.

Aim: This study evaluates the safety, efficacy and eradication of hepatitis C in this subgroup population with SCD.

Methods: 24 patients were recruited from three sickle cell centers in NYC. Inclusion criteria: CHC (Geno specific with variation, diagnosed between 1998–2014) with SCD in remission (with sickle cell history greater than 30 years). All patients were placed LDV 90 mg + SOF 400 mg a day; with food for 12 weeks.

Results: Undetectable, day 7, 14, 30, 60 and 90 days of 9/24, 14/24, 22/24, 22/24 and 22/24 respectively.

Conclusion: This study demonstrates that LDV and SOF combination in SCD patients with CHC is safe and well tolerated; with an SVR12 of 91.67 % (22/24) with 8.3 % (2/24) viral failure (in concomitant genotypes; 1a/4c and 1a/3c).

P-0124

Sofosbuvir, ledipasvir in IBD patients with biologics including ribavirin for HCV: SOLATAIRE C trial

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Background: The prevalence and concomitant treatment of chronic HCV and IBD has not been elucidated. IBD and CHC of greater than 20 years duration may have more fibrosis due to immune suppression. Treatment of IBD often requires biological therapies to achieve longer disease-free remission, which further accelerates higher HCV replication.

Aim: To evaluate the role and efficacy of new NS5A + NS5B inhibitors with and without RBV in treating CHC in moderate to severe IBD requiring biologic therapy.

Methods: 35 patients were recruited from the Kings County Hospital Medical Center, Brooklyn. Inclusion criteria CHC with IBD, Age: greater than 18, HCV Viral Load: greater than 400,000 IU/mL, Genotype 1. Fibrotic Score: Metavir F1 to F4 Primary End Point: SVR12 Secondary End Point: IBD activity index, sustained control of symptoms SVR 24 and complete IBD remission at 30 weeks Group A (n = 17) RBV 1000 mg + LDV and SOF, for 8 weeks Group B (n = 18) LDV and SOF, for 12 weeks.

Results: This study demonstrates the efficacy of LDP and SOF for HCV genotype 1 (94.1–100 % SVR 12) and its safety in the presence of active IBD therapy with TNF Alfa antagonists while keeping the IBD in uninterrupted remission. Both groups had similar SVR 12 (94.1 % in Group A compared to 100 % in group B).

Conclusion: This study demonstrates that DAAs for HCV appear to have similar efficacy and safety in the presence of active IBD therapy with TNF α antagonists while keeping the IBD in uninterrupted remission.

P-0125

No clinically relevant differences in sofosbuvir and ledipasvir exposure across various ethnicities

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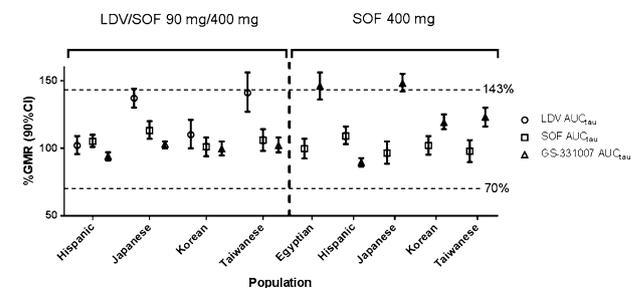
Gilead Sciences, Inc., Foster City, CA, USA

Background: Ledipasvir/sofosbuvir 90 mg/400 mg (LDV/SOF) and SOF 400 mg are approved for treatment of HCV infection in the U.S. and E.U. LDV and SOF are P-gp and BCRP substrates. LDV is primarily excreted in feces unchanged while SOF is metabolized by cathepsin A (CatA) and carboxylesterase 1 (CES1). Ethnic and demographic differences in the exposure of LDV, SOF, and its primary circulating metabolite GS-331007 were explored across regional LDV/SOF and SOF Phase 3 studies.

Methods: Within each ethnic group (e.g. Egyptian, Hispanic, Japanese, Korean, and Taiwanese), LDV, SOF and GS-331007 exposure estimates were generated for each subject with measurable concentrations of LDV, SOF and GS-331007 using previously established population PK models. The effect of demographic variables on LDV, SOF, and GS-331007 exposure was evaluated. Results across ethnic groups were compared to the representative Caucasian U.S. Phase 2/3 population (reference group).

Results: Figure 1 presents %GMR and 90 % CI (ethnic group versus Caucasian U.S. Phase 2/3 population) for LDV, SOF, and GS-331007 AUC_{tau}. Modest increases in the exposure of LDV or GS-331007 (in SOF studies only) across ethnic groups were not considered clinically significant based on exposure-safety evaluations. SOF exposure was unchanged. Similar to findings from the U.S. Phase 2/3 populations, no clinically significant relationships were seen between demographic variables and LDV, SOF, or GS-331007 exposure.

Conclusion: Across multiple ethnic groups, the pharmacokinetics of LDV, SOF, and GS-331007 do not demonstrate clinically meaningful differences.



LDV/SOF studies were not conducted in the Egyptian population.

P-0126

Cost-effectiveness analysis related to HCV treatment is possible to be estimated in each province

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Background: Recently, the HCV therapeutic drug of SVR (sustained virological response) is increased and approximately 100 %. It is expected to contribute to decrease the number of HCV patient. But its economic effect is not evaluated enough. We proposed the cost-effective estimation model based on the liver cancer mortality, the population and QOL scores of each prefecture's.

Methods: To determine QOL scores, 212 patients in Hiroshima University Hospital were asked unsigned questionnaire by Japanese EQ-5D (Mobility, Self-care, Usual activities, Pain and anxiety) during August to September in 2015. The number of patients was estimated by Markov model using disease transition probability. Moreover, incremental cost-effectiveness ratio (ICER) of triple therapy (Peg-IFN + RBV + SMV) and DAA (LDV + SOF) was determined using direct costs (medical costs and treatment costs) and QOL scores in Hiroshima prefecture.

Results: QOL scores of chronic hepatitis, cirrhosis, and hepatocellular carcinoma were 0.871, 0.774, and 0.780, respectively from our survey in Hiroshima. Compared DAA treatment with triple therapy of HCV patients, ICERs with 5, 10 and 20 years analysis duration were estimated as 3910, 202, -2387 (thousand yen/QALY) in men and 477,553, 17,270, -1740 in women, respectively.

Conclusion: According to the estimated ICERs of DAA treatment for HCV patients with QOL score in Hiroshima prefecture, DAA treatment in men found to be cost-effective at 5, 10 and 20 years and that in women only after 20 years. Furthermore, we will continue to simulate the sensitive analysis with the consulting rate and the hepatitis virus check-up rate.

P-0127

Cost-effectiveness analysis of sofosbuvir-based regimens for CHC GT2-real life practice in China

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Background and aim: Sofosbuvir (SOF) is a nucleotide analog inhibitor of hepatitis C virus NS5B polymerase, the key enzyme mediating HCV RNA replication, and its efficacy has been proved in patients with HCV genotype 1, 2, 3 or 4 infection. We aimed to evaluate the cost-effectiveness of SOF-based regimens i.e. 12-week SOF (Sovaldi; Gilead) plus daclatasvir (Daklinza; BMS) (SOF-DCV), 12-week SOF plus ribavirin (SOF-RBV), and 12-week SOF plus ledipasvir (Harvoni; Gilead) (SOF-LDV) compared to SOC in Chinese HCV genotype 2a patients in real life practice.

Methods: By the end of August 2015, our center (Division of Gastroenterology & Hepatology, Humanity & Health Medical Centre, Hong Kong) treated 39 GT2a patients with SOF-based regimens. SVR 12 rate was 100 % for all SOF-based regimens. A Markov model was constructed by treatment history, fibrosis stage and sex based on the real life data. We calculated the incremental cost-effectiveness ratios (ICERs) for each regimen compared with SOC. The robustness of the results was tested by deterministic and probabilistic sensitivity analysis.

Results: SOF-LDV increased 1.6 QALYs resulting ICER of US\$47,234 and US\$37,277 in treatment-naïve and -experienced patients. The probability of cost-effectiveness was 53, 35, and 49 %

for SOF-RBV, SOF-DCV, and SOF-LDV at a willingness-to-pay (WTP) threshold of US\$45,564 (6 times GDP per capita in China) in treatment-naïve patients. The probability was 62, 39, and 52 % correspondingly in treatment-experienced patients.

Conclusion: Due to the high cost of SOF-based treatments, they do not provide good value for money for Chinese patients with HCV genotype 2 infection.

P-0128

Cost-utility analysis of sofosbuvir for the treatment of genotype 2 chronic hepatitis C in Japan

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Background: Current treatment options for CHC have several limitations due to side-effects, interferon ineligibility and intolerance, long treatment durations and low sustained virological responses (SVR) rates, especially for the most severe patients. Sofosbuvir (SOF), administered in combination with ribavirin (RBV), demonstrated high SVR rates of 97 % in Phase III trials across multiple genotypes (GT); it is also the first available treatment regimen for patients that are unsuitable for interferon. However, there are growing concerns regarding cost implications of SOF. Hence, our objective was to conduct cost-utility analysis of SOF + RBV in GT2 patients in Japan.

Methods: A Markov model followed patients until 100 years of age. Approximately 20 % of patients initiated treatment at the cirrhotic stage. Comparators were based on current recommendations in Japan, including pegylated interferon (PEGIFN) with ribavirin (RBV), telaprevir (TVR) in combination with PEGIFN + RBV and no treatment. Quality-Adjusted Life Years (QALYs) was set as the primary outcome measure.

Results: SOF + RBV produced better clinical outcomes than all other regimens did and proved to be cost-effective across all the studied indications, especially in patients unsuitable for interferon, with incremental cost-effectiveness ratios (ICERs) lower than the hypothetical threshold value, or the JPY5Million/QALY gained (Table 1). Results were robust to sensitivity analyses.

Conclusions: SOF in combination with RBV was shown to be cost-effective in GT2 patients in Japan. Compared to PEGIFN + RBV, TVR + PEGIFN + RBV and no treatment, SOF + RBV offers a more efficacious, shorter and better tolerated treatment option as well as treatment for IFN-ineligible patients

Table 1. Summary of results per indication

Indication	Comparator	Difference total direct costs	QALY gained	CC avoided	DCC avoided	HCC avoided	LT avoided	ICER (JPY/QALY)	ICER (JPY/QALY)
									with indirect costs included
GT2 TN UI	No treatment	-864,643	6.63	6,485	3,382	1,301	35	Dominant	Dominant
	PEGINF2b + RBV (24 wks)	2,889,064	1.34	1,330	679	402	7	2,148,465	Dominant
GT2 TE UI	No treatment	-695,366	6.51	6,381	3,316	622	34	Dominant	Dominant
	PEGINF2b + RBV (48 wks)	1,082,154	2.19	2,207	1,128	2,723	11	494,049	Dominant
	TVR + PEGINF2b + RBV	2,787,033	0.78	1,005	403	4,846	4	3,540,914	Dominant
(non-cirrhotic only)									

GT: Genotype; HCC: Hepatocellular carcinoma; ICER: Incremental cost effectiveness ratio; IE: Interferon eligible; LT: Liver transplant; Lys: Life years; PEGINF2b: Pegylated interferon alpha-2b; QALYs: Quality adjusted life years; RBV: Ribavirin; TE: Treatment-experienced; TN: Treatment naïve; TVR: Telaprevir; UI: Unsuitable for interferon

*P-0129***Effect of sofosbuvir and ribavirin treatment in HCV genotype 2 Japanese patients**

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We evaluated therapeutic effect of SOF + RIB in HCV infected chronic liver disease in our hospital. 57 cases with Genotype 2 HCV infected patients treated with SOF + RBV were enrolled (28 male, 29 female, average age 63 y.o. and average weight 59.3 kg). Average HCV RNA levels before treatment start was 5.8. Naive patients were 47 patients, and previous treatment patients were 10. All patients were treated with 400 mg dose of SOF and RIB from 400 to 800 mg by individual body weight.

Results: HCV-RNA average levels was 5.78 at before treatment, 0.93 at 2 weeks, 0.26 at 4 weeks. And all patients HCV-RNA were disappeared at 6 weeks. Virus disappearance rate became 41.2 % (21/51) at 2 weeks, 82.0 % (41/50) at 4 weeks and 100 % (46/46) at 6 weeks. Disappearance rate of HCV-RNA at 4 weeks later (RVR) was 88.5 % (23/26) in males and 75 % (18/24) in female, 75 % (18/24) less than 64 years old and 88.5 % (23/26) more than 65 years old, 80.5 % (33/41) in chronic hepatitis patients and 88.9 % (8/9) in cirrhosis patients. Serum hemoglobin levels decreased from 13.9 to 12.9 at 2 weeks and 12.1 at 4 weeks. And serum ALT levels significantly decreased from 65.4 to 21.1 at 2 weeks, 19.7 at 4 weeks. Serum AFP levels also decreased from 34.0 to 13.7 at 2 weeks, 6.8 at 4 weeks. There were no severe adverse events during treatment.

Conclusion: Treatment of SOF + RBV for HCV genotype 2 infected Japanese chronic liver disease patients was more safety and well tolerable and well effective than IFN based therapy.

*P-0130***Efficacy and side effects of SOF/RBV in patients with hepatitis C virus genotype 2 infection**

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Background and aims: Sofosbuvir and ribavirin therapy (SOF/RBV) was approved for the treatment of chronic hepatitis and cirrhosis with genotype 2 hepatitis C virus (HCV) on May 2015 in Japan. Compared to interferon (IFN) based therapy, higher sustained virological response (SVR) rate and fewer side effects are expected. We examined the response rate and side effects of SOF/RBV.

Methods: Since May 2015, we started SOF/RBV in 39 patients (22 males and 17 females) with chronic hepatitis C and cirrhosis. Age is 67 ± 11 years. Patients with liver cirrhosis and those with hepatocellular carcinoma were 21 % (8/39) and 5 % (2/39), respectively. HCV genotype was 2a in 20 patients, 2b in 12, and undetermined in 7.

ITPA gene SNP (rs 1127354) is AA in 1 patient, AC in 2, CC in 16, and undetermined in 20.

Results: HCV-RNA negative rate was 57 % (16/28) at 2 weeks, 76 % (22/29) at 4 weeks, 100 % (21/21) at 8 weeks, 100 % (19/19) at 12 weeks, and 100 % (6/6) at 4 weeks after cessation of SOF/RBV. There were no discontinuations due to side effects. The reduction of hemoglobin at 4 weeks were significantly larger in the patients with ITPA genotype CC (2.1 ± 1.2 g/dl) than those with non-CC genotype (0.4 ± 0.3 g/dl) (p = 0.0333).

Conclusions: SOF/RBV is highly effective to make HCV-RNA negative. Occurrence of anemia significantly less frequently occurred in the patients with ITPA SNP of non-CC. Further follow-up is necessary to confirm efficacy and side effects of SOF/RBV.

*P-0131***Sofosbuvir plus ribavirin treatment for HCV-infected patients with ulcerative colitis**

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Direct-acting antivirals (DAAs) are now available for the treatment of chronic hepatitis C virus (HCV) infection. Interferon-free regimen seems useful, safe and effective for many patients, who are contraindication for interferon-based treatment. We experienced a 56-year-old treatment-naive man with chronic HCV 2b infection who also had ulcerative colitis and dilated cardiomyopathy. He was treated with sofosbuvir (400 mg daily) and ribavirin (600 mg daily). HCV RNA levels were 6.3 LIU/mL, 2.5 LIU/mL, 1.4 LIU/mL, and 0 LIU/mL before and 1, 2, and 4 weeks after the commencement of treatment. At 4 week, exacerbation of ulcerative colitis with diarrhea and bloody faces was observed. At 7 week, diarrhea and bloody faces was more frequently. After ribavirin was reduced to 400 mg daily, these symptoms were decreased. He is now continuing to receiving treatment safely at 10 week. We experienced HCV-infected patients with exacerbation of ulcerative colitis and the reduction of ribavirin was effective for the improvement of his symptom during sofosbuvir plus ribavirin regimen. Clinician might pay careful attentions for the ribavirin dose in the treatment of certain HCV-patients with inflammatory bowel diseases such as ulcerative colitis, in the treatment of sofosbuvir plus ribavirin regimen.

*P-0132***Efficacy of sofosbuvir/ribavirin for Japanese patients with HCV genotype 2 infection**

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Background and aim: In Japan, combination therapy of NS5A RNA dependent RNA polymerase inhibitor Sofosbuvir (SOF) and Ribavirin (RBV) for patients with hepatitis C virus (HCV) genotype 2 infection

has been approved clinically in June 2015. This therapy improved efficacy and was well tolerated in phase III trial. We conducted a prospective study to investigate the efficacy and the safety of SOF/RBV therapy for Japanese patients with HCV genotype 2 infection. **Method:** Total of 72 patients with HCV genotype 2 who received orally SOF 400 mg once daily plus RBV twice daily the dosage determined according to body weight (600–1000 mg) for 12 weeks at our hospital were enrolled in this study. There were 37 patients with genotype 2A and 35 patients with genotype 2B. The patients included 54 men and 18 women, whose age ranged from 17 to 84 years (mean \pm SD: 62.0 \pm 14.5). HCV resistant associated variants (RAVs) were analyzed by direct sequence of the HCV NS5B domains.

Results: The population of the patients having liver cirrhosis and history of hepatocellular carcinoma were 36 and 24 %, respectively. No HCV RAV of NS5B domain was present in the patients. SOF/RBV decreased rapidly HCV-RNA level. In all patients HCV-RNA became under a lower limit of quantification of 25 IU/mL within 4 weeks. Several patients required dose reduction of RBV due to anemia or fatigue. However, no patients discontinued the therapy.

Conclusion: SOF/RBV for Japanese patients with HCV genotype 2 had high effectiveness and was well tolerated.

P-0133

Outcomes of SOF and RIBA therapy for HEP C G3 patients who are relapsers of PEG INF and RIBA

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Background and aims: Sofosbuvir + Ribavirin is gold standard for chronic HEP-C patients. We evaluated the safety and efficacy of sofosbuvir and ribavirin in chronic HEP-C G3 patients.

Methods: We prospectively enrolled 150 patients with chronic HEP-C G3. These patients were relapsers/non responders with previous therapy of peg inf and Riba. Patients with de compensated cirrhosis, platelets count <100, portal vein size >13 mm on ultrasound were excluded from this study. Eligible patients were enrolled to receive Sovaldi 400 mg daily and Ribavirin 10 mg/Kg body weight for 6 months. These patients were seen in outpatient 4 weekly. They had baseline CBC, TSH, Blood Sugar, HCV Genotype and HCVPCR quantitative. Each patient had CBC at every 4 weeks. HCVPCR at week 4, 12 and 24.

Results: This is our ongoing study we are presenting interim analysis of 100 patients who have completed 24 weeks of treatment. Out of 100 patients 75 % were male, 25 % were female, median age 48. 98 patients out of 100 (98 %) were PCR negative at week 4, 12 and 24 respectively. Two patients did not have PCR Negative after 8 weeks of treatment thus treatment was stopped. All patients are under regular review to monitor HCVPCR 6 months after stopping the treatment (SVR). The most common AEs were nausea, fatigue, and dizziness.

Conclusions: Sof + Riba for HCV-G3 patients is very effective treatment. Most patients achieved RVR without any major side effects. In our study response rate after completing 24 months of treatment was 98 %. Pretty safe safety profile.

P-0134

Rapid decrease of the non-invasive serum liver fibrosis marker WFA⁺-M2BP by IFN-free therapy

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Background and aims: The *Wisteria floribunda* agglutinin-positive human Mac-2-binding protein (WFA⁺-M2BP) is a novel serum liver fibrosis glycoprotein marker. Consistently elevated post-treatment serum WFA⁺-M2BP levels have been reported to be associated with the development of HCC after sustained virological response by chronic hepatitis C patients. However, it is not yet known when and to what extent the WFA⁺-M2BP level decreases with IFN-free anti-viral treatment. This prospective study was done to evaluate the serial change of WFA⁺-M2BP during the treatment of chronic hepatitis C patients.

Methods: The data of 98 genotype 2 hepatitis C patients who received sofosbuvir and ribavirin was available for analysis. The serum WFA⁺-M2BP level was measured pre-treatment and at weeks 1, 2, 4, 8, and 12 after starting therapy.

Results: The serum HCV-RNA levels of all patients rapidly declined with decreasing AST and ALT. The mean serum WFA⁺-M2BP level was significantly decreased at 1 week, from 2.81 to 2.05 COI, $P < 0.0001$. The mean WFA⁺-M2BP level progressively decreased to 1.89, 1.79, 1.62, and 1.62 COI at weeks 2, 4, 8, and 12, respectively. When the patients were divided into four groups by pre-treatment serum WFA⁺-M2BP level (<1.0, 1.0–2.0, 2.0–4.0, and >4.0), the rates of decrease were –7.9, 29.1, 38.8, and 31.5 % at week 1 and 26.6, 40.8, 45.6, and 52.0 % at week 12.

Conclusions: The WFA⁺-M2BP level decreased rapidly during week 1 after the start of IFN-free therapy, then continued to decrease slowly to week 12, which indicates a possible prophylactic effect on HCC by IFN-free therapy.

P-0135

Sofosbuvir and ribavirin for chronic hepatitis C: interim analysis from a real-life Indian cohort

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Background: Combination of Sofosbuvir (SOF) and Ribavirin (RBV) for treatment of chronic HCV has improved sustained virological response (SVR) rates in recent trials. Sofosbuvir, a pangenotypic, potent NS5B inhibitor has recently been approved in India (March, 2015). Data concerning the efficacy and safety of this regimen in real-life Indian cohort is lacking.

Methods: A cohort of 298 consecutive chronic HCV patients treated at ILBS, New Delhi. Treatment protocol involved combination SOF

(400 mg/d) and weight based RBV (600–800 mg/d <75 kg and 1000–1200 mg >75 kg body wt) for 24 weeks.

Results: Of 298 patients, 151 had completed 24 weeks of therapy and were analyzed. 111 (73.5 %) had cirrhosis (11.9 % had pre-treatment CTP >7) and 57 (37.7 %) were treatment experienced. Among 114 (75.5 %) patients with Genotype 3 and 35 (23.2 %) with Genotype 1, SVR4 rates were 87.9 % (n = 33) and 77.8 % (n = 9) respectively with lower SVR4 rates in treatment experienced (78.9 vs. 91.3 %) and cirrhotics. Virological response (VR) during treatment was similar irrespective of HCV genotypes, treatment history or cirrhosis (VR at week 1:8–20 %; week 4:70–85 %; week 12:95–100 %; week 24:98–100 %). Six patients with relapse had slower VR during treatment at week 1(0 vs. 12.5 %) and week 4(50 vs. 80.6 %). None had major adverse effects requiring discontinuation of therapy. Anemia, raised bilirubin and myalgia were more frequent in cirrhotics.

Conclusions: In a real-life Indian cohort, preliminary results showed a high rate of virological response with SOF-RBV based treatment regimes. However, larger and longer post-treatment follow up is required to know the relapse rates, specially in difficult-to-treat patients including treatment experienced cirrhotics.

P-0136

Sofosbuvir plus ribavirin for HCV genotype 2a reinfection after LDLT

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Living donor liver transplantation (LDLT) is one of the therapeutic option for patients with end-stage liver diseases associated with HCV infection. But HCV reinfection after liver transplantation is one of the difficult-to-cure situations. Compared with interferon-based regimen, interferon-free regimen against HCV infection is more effective and safe treatment with less adverse events. We report a 66-year-old woman with HCV genotype 2a reinfection after LDLT, who were successfully treated with sofosbuvir plus ribavirin. She received sofosbuvir plus ribavirin approximately 5 months after LT although she had IL28B favorable alleles. After the commencement of sofosbuvir plus ribavirin, HCV RNA levels became <1.2 LIU/mL and negative at 1 week and 4 week. She received sofosbuvir plus ribavirin for 12 weeks. She had sustained virologic response at 4 week after stopping treatment. Sofosbuvir plus ribavirin seems an excellent treatment with less adverse events for HCV genotype 2 infection, but cautious attention and careful follow-up should be needed.

P-0137

100% Efficacy of hepatitis C treatment in cirrhotic patients: real life results in Latvia

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Background: The interferon-containing regimens for the treatment of hepatitis C virus (HCV) infection are associated with significant side effects and reduced efficacy especially in patients with cirrhosis. The aim of this study was to evaluate efficacy of the interferon-free combination of the protease inhibitor paritaprevir with ritonavir the NS5A inhibitor ombitasvir the non-nucleoside polymerase inhibitor dasabuvir, and ribavirin in patients with genotype 1 infection and compensated cirrhosis.

Material and methods: 15 patients, 6 men and 9 women, at the age from 39 to 70 with HCV genotype 1b infection and compensated cirrhosis (METAVIR 4), Child Pugh A were included in the study. 10 patients were peginterferon and ribavirin treatment experienced, 5-treatment naïve. Patients receive 12 weeks treatment with paritaprevir/ritonavir/ombitasvir at a once-daily dose of 150 mg/100 mg/25 mg, dasabuvir (250 mg twice daily), and ribavirin administered according to body weight. The primary efficacy end point was a sustained virologic response (SVR) 12 weeks after the end of treatment.

Results: 15 patients out of 15 included achieved SVR at post-treatment week 12, for a rate of 100.0 %. In total three non-clinically significant adverse events were reported: insomnia, anemia and itch. All patients completed treatment regimen course. None of patients discontinued treatment due to adverse events.

Conclusions: Interferon-free combination of paritaprevir/ritonavir/ombitasvir, dasabuvir and ribavirin is highly effective and well tolerated in so difficult to treat patient group as patients with HCV related cirrhosis.

P-0138

RVR response in HCV related decompensated cirrhosis with sofosbuvir in Bangladesh

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Aim: Aim was to assess safety and efficacy of world's first generic sofosbuvir and generic ribavirin in hepatitis C virus (HCV) related decompensated cirrhosis.

Methods: Nine patients were included in the study. Informed, written consent was obtained from all. There were 6/9 (66.7 %) males and 3/9 (33.3 %) females. They were treated with tablet sofosbuvir (400 mg) (Tab. Hopenavir, Incepta Pharmaceuticals, Bangladesh) once daily in combination with generic capsule ribavirin (200 mg) 1000 mg daily in two divided doses for 48 weeks. Tab. Hopenavir used in this study is world's first generic sofosbuvir introduced in Bangladesh in February 2015.

Results: All had HCV genotype 3 except 1 with genotype 1 and 1 having genotype 4. Five completed 4 weeks treatment at data analysis, while 1 patient expired from hepatic encephalopathy. Four out of

5 had rapid virological response (RVR) (HCV RNA <20 IU/ml). At baseline their HCV RNA was 1019–3,700,000 IU/ml (mean 12,376,898.8 IU/ml). No significant adverse event was noted in any and no dose reduction was necessary.

Conclusion: Initial experience with generic anti-virals including world's first generic sofosbuvir appears to be safe and promising. Definite comment can be made once all patients are evaluated at 12 weeks off treatment.

P-0139

Sofosbuvir plus daclatasvir with ribavirin for 12 weeks in Chinese refractory hepatitis C patients

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Background: The antiviral therapy for chronic hepatitis C has changed to the era of direct-acting antiviral agents currently, unfortunately, the clinical data for Chinese patients is lacking because the unavailability of the agents in China. Direct-acting antiviral agents are urgent for some Chinese refractory patients, i.e., treatment-experienced cirrhotic patients infected with genotype 1 hepatitis C virus. **Methods:** 5 treatment-experienced cirrhotic genotype 1b hepatitis C patients were administered with sofosbuvir/daclatasvir/ribavirin for 12 weeks. The primary efficacy endpoint was the sustained virologic response (SVR), which was defined as an undetectable HCV RNA level at 12 and 24 weeks (SVR 12 and SVR 24) after the cessation of therapy. Adverse events that occurred during and after therapy are also documented.

Results: We observed that this triple therapy is safe and well-tolerated for Chinese patients, 5 (100 %) cases obtained SVR 12 and SVR 24, and liver stiffness decreased in 4 (80 %) cases at SVR 24.

Conclusions: This study firstly presents the safety and efficacy information of 12 weeks of sofosbuvir/daclatasvir/ribavirin administration for Chinese treatment-experienced genotype 1 patients with cirrhosis, which is different from the 24 weeks of treatment duration that European Association for the Study of the Liver recommended, and further indicates that East Asia hepatitis C populations may be easier to treat compared to western populations in current DAAs era. Future validations with large number of cases are needed.

P-0140

A case of liver dysfunction without an elevation of ALT caused by a treatment of DCV and ASV

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A 78-year-old female with hepatitis C was started on a planned 24 week course of Daclatasvir (DCV) and Asunaprevir (ASV) treatment. She was a treatment-naïve case, and had compensated cirrhosis. 15 days after starting treatment, she had general malaise, and her blood test showed a striking elevation in total bilirubin, and prolonged prothrombin time. However, there was no elevation in

AST or ALT. Her abdominal CT showed a few ascites and Rt. mild pneumonia. She was diagnosed with hepatic failure and immediately hospitalized. Further blood examination showed eosinophilia and a slight elevation of serum IgE and CRP. Drug-induced lymphocyte stimulation test for both DCV and ASV was positive. She was diagnosed with acute liver failure due to drug-induced liver injury. The DCV/ASV therapy was discontinued on day 16 after starting treatment. After discontinuation of the therapy, her hepatic functional reserve improved, and she attained her serological viral response (SVR) 4 weeks after the treatment. We hypothesized that there were two mechanisms of this elevation in total bilirubin. The first is type I hypersensitivity. Her blood tests definitely showed the involvement of this allergy. The second is the dysfunction of transporters. The drug instructions say that Daclatasvir is a moderate inhibitor of OATP and P-gp in hepatocytes. Rt. Pneumonia might have worsened liver dysfunction including these transporters. There is no report about a striking elevation in total bilirubin without elevation of ALT in the post-marketing surveillance by BMS. We report this case in detail in conjunction with all the cases underwent in our department.

P-0141

Adverse effects of combined administration of daclatasvir and asunaprevir

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Background: Measures against adverse effects of direct-acting antivirals (DAAs) are important because DAAs are administered to elderly patients or patients with liver cirrhosis (LC) in actual clinical settings. The occurrence of the adverse effects in the early stage of the introduction of DAAs therapy was examined.

Subjects: One hundred and seventy-five patients who were treated with daclatasvir (DCV) and asunaprevir (ASV) 12 weeks previously were enrolled in this study (The mean age : 72 ± 10.2 years, males/females : 65/100, chronic hepatitis (CH)/LC: 117/58).

Results: Eighty-two (46.8 %) patients developed adverse effects and 12 (2.8 %) patients discontinued the agent. Major subjective symptoms of adverse effects include pharyngeal pain, fever, headache, rash, and itching sensation. Elevated levels were found for ALT, eosinophil counts, creatinine, uric acid, and creatine phosphokinase. Among 12 patients who discontinued the agent, five patients had poor systemic conditions with fever within 21 days after the start of administration; decreased liver function and elevated biliary enzyme levels were observed. In addition, most of them had elevated eosinophil counts. In the remaining seven patients, elevated ALT levels of Grade 4, systemic rash, and rhabdomyolysis were observed. Six of the patients who discontinued the therapy achieved a sustained virological response at post-treatment week 12 (SVR12).

Conclusions: Owing to the adverse effects with subjective symptoms, many patients were required to reduce the dosage or discontinue the administration of DCV and ASV. It is considered that the criteria for the reduction or discontinuation of administration should be discussed and clarified.

P-0142

Efficacy and adverse events of daclatasvir/asunaprevir in chronic hepatitis C genotype1b

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Background/aims: Interferon-free daclatasvir (DCV) and asunaprevir (ASV) therapy for 24 weeks improved efficacy for patients with hepatitis C virus (HCV) infection. The aim of this study is to assess the treatment outcome according to resistance associated variants (RAVs) in NS5A regions and adverse events.

Methods: 680 patients were enrolled at multi centers in Japan: 449 chronic hepatitis and 231 liver cirrhosis patients. The median age was 71(25–87) and male was 305(44.8 %).

Results: Direct sequencing analysis of 612 patients administered DCV/ASV therapy showed the existence of 31M (1.4 %), 93H (4.2 %), and 31 M/93H (0.3 %) variants in NS5A region. Comparing to undetectable HCV-RNA rates with and without 93H mutation at baseline, week 4/week 8/week 12/ETR/SVR4/SVR8/SVR12 were 81.4/97.0/97.7/92.6/90.9/85.7/82.8 (%) in 93Y group, whereas 53.8/83.3/95.5/73.9/65.2/60.9/56.5 (%) in 93H group, suggesting that undetectable HCV-RNA rate was significantly lower in patients with 93H mutation. In 93Y group, 53.6 % (15/28) had treatment failure among patients with prior triple therapy including SMV failure. Multiple RAVs such as L31 M/Q54H/Y93H in NS5A were emerged by treatment failure. The number of RAVs were 0/1/2/3 = 81/19/0/0 (%) at baseline, and 0/4/52/37 (%) at the time of virologic failure; patients with multiple RAVs increased from 0 to 89 %. Fourteen (2.0 %) cases stopped treatment due to ALT elevations (>300 U/l). Seven of 14 discontinuation cases were measured serum ASV concentrations and percentage of eosinophil 4 weeks before ALT elevation.

Conclusion: History of SMV therapy and pre-existing NS5A Y93H were associated with virologic failure during DCV/ASV therapy, resulting in emergence of multiple RAVs. Hence, patients with RAVs at baseline will be required assessment for optimizing future DAAs therapies.

P-0143

Frequency and severity of liver injury during daclatasvir and asunaprevir dual therapy for HCV

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Background: To clarify the frequency and severity of liver injury during daclatasvir and asunaprevir dual therapy for hepatitis C virus (HCV) infection.

Method: A total of 123 patients infected with HCV genotype 1 were included (mean age of 67 years, male:female = 58:65, chronic hepatitis:compensated cirrhosis = 79:44). All patients were treated with daclatasvir (60 mg/day) and asunaprevir (200 mg/day).

Results: The undetectable rate of HCV RNA at the end of treatment was 95 %. In the majority of patients, transaminase levels decreased and fell below the upper limit value 4 weeks after starting the treatment. During the treatment, the percentages of patients whose transaminase levels exceeded the upper limit were as follows: AST, 28 %; ALT, 20 %. AST elevation emerged not only during the first half of treatment (5–8 weeks, 31 %; 9–12 weeks, 34 %), but also during the last half (13–16 weeks, 11 %; 17–20 weeks, 11 %; 21–24 weeks, 9 %). Approximately 21 % of the patients whose AST levels exceeded the upper limit showed an increasing AST level over 3 times the upper limit at a mean of 12 weeks and required a dose reduction in asunaprevir by half or discontinuation of the treatment. Eight patients discontinued therapy before 24 weeks due to liver injury (3 patients), viral breakthrough (2 patients), fatigue (2 patients) and thrombopenia (1 patient).

Conclusions: To avoid the progression of severe liver injury, monitoring of transaminase levels is necessary during daclatasvir and asunaprevir dual therapy.

P-0144

A single dose of RG-101 results in undetectable HCV-RNA levels in chronic hepatitis C patients

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Aim: The aim of this study was to evaluate the safety and efficacy of a single dose of RG-101, a carbohydrate conjugated oligonucleotide that targets miR-122 in hepatocytes, in chronic HCV patients.

Methods: In this multicenter phase 1 study, we included 32 chronic HCV patients with genotype 1 (n = 16), 3 (n = 10) or 4 (n = 6) infection. Patients received a single subcutaneous injection of 2 (n = 14) or 4 mg/kg RG-101 (n = 14), or placebo (n = 4). All patients were followed 8 weeks after randomization, and patients with >2 log decrease in HCV RNA level from baseline and <1 log increase from nadir were included in an extended follow-up study in which patients were followed until 28 weeks after dosing. HCV RNA levels were measured using Roche COBAS AmpliPrep/COBAS Taqman HCV v2.0 assay.

Results: At baseline, mean HCV RNA levels were comparable between RG-101 dosed patients versus placebo (6.2 versus 6.4 log 10 IU/mL). The mean viral load reduction at week 4 was 4.4 log 10 IU/mL (range 2.3–5.8) in patients dosed with RG-101 (p < 0.001). At week 8, 22/28 patients dosed with RG-101 were included in the extended follow-up. At week 28, 13/22 patients experienced a virological rebound, 3/22 patients were lost to follow-up and 6/22 patients

(gt 1 n = 1, gt 3 n = 2, gt 4 n = 3) had undetectable HCV RNA levels.

Conclusion: A single administration of 2 mg/kg or 4 mg/kg RG-101 resulted in undetectable HCV RNA levels in 6 patients with various HCV genotypes at week 28 of follow-up.

P-0145

CC-31244, a pan-genotypic, potent HCV NS5B non-nucleoside polymerase inhibitor

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Cocrystal Pharma Inc.

Purpose/background: NS5B non-nucleoside inhibitors (NNI) are a distinct class of direct acting agents (DAA) for the treatment of HCV. We designed and characterized a novel, pan-genotypic NNI (CC-31244), which is targeted for use in combination DAA therapies. We present here the structure-based drug design approach, in vitro characterization, resistance profiles, and pharmacokinetic profiles of CC-31244.

Methods: NS5B polymerases (GT1-6) and drug resistant NS5B polymerases were purified for protein crystallization and IC50 determination. Antiviral activity and pharmacokinetic properties were determined.

Results: CC-31244 showed pan-genotypic activity against genotypes 1a–6a (EC50 2–26 nM). High resolution X-ray data have confirmed that CC-31244 binds to a highly conserved drug binding pocket, NNI-4, and extends to the highly conserved active site of the NS5B polymerase. CC-31244 showed excellent activity against the NNI-4 drug resistant variants including S365T and C316Y. HCV replicons with reduced susceptibility to CC-31244 have been selected in cell culture for GT1b.

Conclusion: A pan-genotypic NNI lead, CC-31244, demonstrated potent HCV antiviral activity, favorable in vitro safety, and drug resistance profiles. CC-31244 has been selected to advance to Phase 1 clinical studies in 2016.

P-0146

Chinese ethnicity does not result in clinically relevant differences in sofosbuvir pharmacokinetics

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Aim: Sofosbuvir (SOF) is approved in the United States and European Union for the treatment of chronic hepatitis C infection. In support of ongoing clinical development in China, a Phase 1 single and multiple dose study was conducted in healthy Chinese subjects to evaluate the safety, tolerability and pharmacokinetics of SOF 400 mg. Exposures of SOF, and its predominant circulating metabolite, GS-331007, in Chinese subjects were compared to exposures previously observed in a representative healthy Caucasian subject population

Methods: Single and multiple (7 days, once daily) doses of SOF 400 mg were administered under fasted conditions. Plasma PK parameters AUCinf (single dose), AUCtau (multiple dose), and Cmax (single and multiple dose) were calculated for SOF and GS-331007 (Table 1). Geometric Mean Ratios (%GMRs and 90 % CIs) were calculated for AUCinf and Cmax (Single Dose Chinese/Caucasian). Exposures were considered pharmacokinetically equivalent if the 90 % CI of the %GMR was within 70–143 %.

Results: Fourteen Chinese subjects were enrolled and completed the study. SOF 400 mg was safe and well tolerated. SOF and GS-331007 showed minimal to no accumulation upon multiple dosing (Table 1). GS-331007 exposures were similar between the two populations. SOF exposure modestly increased (41–62 %), but was not considered clinically significant based on the known safety-exposure relationships of SOF.

Conclusions: No clinically relevant differences in the PK of SOF or GS-331007 were observed between Chinese and Caucasian subjects, supporting the appropriateness of the 400 mg daily SOF dose in the Chinese population.

Parameter, Mean (%CV)	Chinese Population		Reference Population	%GMR (90% CI) Chinese Reference Single Dose
	Single Dose (N=14)	Multiple Dose (N=14)	Single Dose (N=59)	
SOF				
AUC (ng·h/mL)	861 (37.6)	872 (47.4)	629 (44.9)	141 (116, 171)
C _{max} (ng/mL)	1000 (46.3)	922 (39.1)	622 (36.1)	162 (126, 208)
GS-331007				
AUC (ng·h/mL)	10,200 (17.4)	10,900 (23.6)	11,100 (22.6)	93.2 (84.6, 103)
C _{max} (ng/mL)	1100 (23.8)	1280 (26.2)	1110 (28.0)	100 (88.2, 115)

Plasma PK parameters are presented to three significant digits.

P-0147

Rapid antiviral response with TG-2349 plus PegIFN-RBV in naive GT-1b subject: an interim analysis

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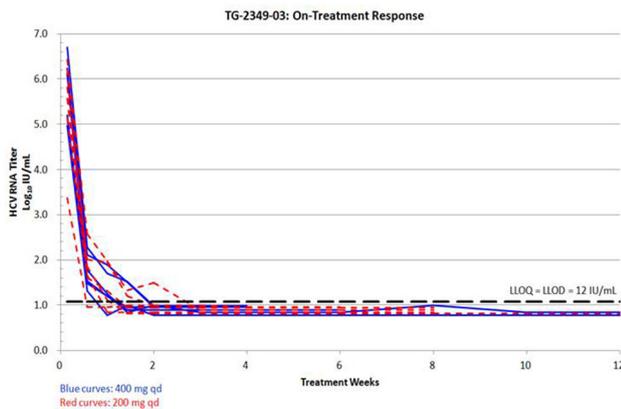
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TG-2349 (Furaprevir) is a novel HCV protease inhibitor under phase II development. In a 3-day monotherapy study at dosages of 200 mg to 600 mg QD, maximal viral load reductions of 3.55 to 4.39 log were observed in GT-1 subjects. Follow up treatment with standard PegIFN/RBV was recommended, taking advantage the steep HCV drop obtained, to six GT-1b subjects after completed 1-week follow up. All

6 patients took SOC achieved RVR and EVR, suggested TG-2349 based triple therapy could improve outcome and reduce treatment durations. TG-2349-03 is a multicenter, randomized, open-label study to evaluate the safety and efficacy of TG-2349 combined with PegIFN/RBV for treatment naive, non-cirrhotic, GT-1b CHC subjects. Study Part A evaluates triple combination for 12 weeks (at 200 and 400 mg qd), with or without an additional 12-week treatment of PegIFN/RBV, based on week-4 virologic response. A shorter 8-week treatment (B) may start based on part A results. The Part A interim analysis is reported here. To date, 16 subjects have been enrolled, including 12 reached treatment week 4. RVR of 100 % was achieved as HCV RNA < LLOQ (12 IU/mL) were observed for everyone. Each subject thus qualified for 12 weeks of total treatment, based on protocol criteria. Three subjects have completed the 12-week treatment, with HCV undetectable at end of treatment and 4-week post-treatment visit. The 12-week TG-2349 + PegIFN/Rbv was safe and well tolerated. AEs reported to date were mild and consistent with the observations associated to PegIFN & RBV. Further development of TG-2349 is warranted

Number of subjects with HCV RNA titer below LLOQ

Treatment Time	Wk1	Wk2	Wk3	Wk4	Wk8	Wk12	SVR4
n/Evaluable N, %	3/13 23%	11/12 92%	12/12 100%	12/12 100%	6/6 100%	3/3 100%	3/3 100%



P-0148

TG-2349 3-day monotherapy with significant antiviral activity in Caucasian and Asian HCV Subjects

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TG-2349 (Furaprevir) is a novel HCV protease inhibitor under phase II development for the treatment of chronic hepatitis C infection. The

phase I study in healthy subjects demonstrated that TG-2349 is safe and well tolerated at single oral dose up to 800 mg and multiple doses up to 600 mg (5 days). Its PK profile supports once daily dosing. Here, we report phase IIa study of 3-day monotherapy with TG-2349. Twenty naive GT-1 subjects were enrolled in the United States and Taiwan. All doses of TG-2349 demonstrated significant antiviral activity. The mean maximal reductions in HCV RNA were 3.55, 3.64, and 3.95 log₁₀ IU/ml in Caucasian GT-1a patients receiving 200, 400, and 600 mg TG-2349, respectively. Log reductions of 3.98 to 4.39 were observed in Taiwanese GT-1b subjects under the same conditions. Thirteen patients infected with GT-2, 3, 4 or 6 were enrolled. Dosage of 600 mg QD for 3 days was given. Mean maximal HCV RNA log reductions were 2.80 (GT-2), 3.53 (GT-4), and 3.58 (GT-6). Limited antiviral activities were observed for GT-3 subjects. No viral breakthrough during dosing period observed except one with GT-2a. Good safety profile was observed. All AEs were grade 1 or 2 except 1 SAE of bacteremia (GT-1, 200 mg) that was drug not related. Most common reported AEs were dizziness, headache, and dehydration. No deaths or discontinuations due to AEs. The results of this phase IIa proof-of-concept study in treatment-naive patients suggest that TG-2349 can be effective in treating HCV GT-1/2/4/6 infections.

P-0149

Serum extracellular matrix degradation inhibitors across stages of liver fibrosis in chronic HCV

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Objective: Tissue inhibitor metalloproteinase-1 (TIMP-1) and alpha-2 macroglobulin (AMG) are extracellular matrix degradation inhibitors that have been demonstrated to increase with liver fibrosis. This study analyses their detailed serum profile across liver fibrosis stages in chronic hepatitis C (CHC).

Methods: Serum TIMP-1 and AMG measurements were evaluated for 78 adult male CHC patients versus liver fibrosis (F) stages (METAVIR F0-F4). The performance characteristics for discrimination of sequential (close stages), significant (F > 2), and advanced (F > 3) fibrosis were assessed.

Results: Both TIMP-1 and AMG correlated significantly with fibrosis (r = 0.31, p = 0.005; r = 0.37, p = 0.001, respectively), but failed to discriminate sequential stages. For discrimination of significant fibrosis, the areas under receiver operating characteristics curves were small (0.59 and 0.57, respectively). At a cut-off value of 743 ng/ml, TIMP-1 showed a 100 % specificity (with 17.6 % sensitivity), while at a cut-off of 3 gm/l, AMG showed 73.5 % sensitivity (with 36.4 % specificity). A similarly modest discrimination was noted for advanced fibrosis. Interestingly, AMG showed an early rise with significantly higher values in F0 compared with healthy controls (3.6 ± 1.1 vs. 1.8 ± 0.6, respectively).

Conclusions: Neither TIMP-1 nor AMG could discriminate the sequential stages of fibrosis. Their modest performances for discrimination of significant and advanced fibrosis are related to the wide normal range of TIMP-1 and the early rise of AMG. A longitudinal monitoring would give a better understanding of their true changes, and examine whether patients having high AMG levels at F0 would be fast fibrosers or respond differently to therapy.

P-0150

A case with hepatitis C undergoing kidney transplantation after telaprevir containing triple therapy**Safak Kaya¹, Nurettin Ay², Birol Baysal³, Senol Comoglu⁴**¹Gazi Yasargil Training and Research Hospital, Diyarbakir, Turkey; ²Gazi Yasargil Training and Research Hospital, Diyarbakir, Turkey; ³Bezmialem University Faculty of Medicine; ⁴Umraniye Training and Research Hospital, Istanbul, Turkey

HCV infection may lead to graft loss in patients who received transplantation for ESRD. We here present a patient with HCV infection, who received haemodialysis, were candidates for kidney transplantation and were treated with short time telaprevir triple therapy due to side effects. A 43-year-old male patient presented to the transplantation unit to be listed for kidney transplantation. His HCVRNA was 392,000 IU/ml and HCV was genotype 1a. The patient was started on subcutaneous pegylated interferon alpha-2a at 135 mcg once a week, oral ribavirin at 200 mg/day three times a week on dialysis days and oral telaprevir at 750 mg three times a day. He was hospitalized 2 weeks later for nausea, vomiting and oral nutritional deficiency. He was given intravenous fluid replacement and antiemetic therapy. The treatment was maintained in the meantime. Because improvements were achieved in his complaints, he was discharged a week later with the decision to continue with the treatment. One week after discharge, the patient was admitted for inpatient care for even further worsening of the complaints. With no improvements in symptoms, treatment was withdrawn at week 6. HCVRNA negative in follow-up measurements at months 3, 6 and 9 following treatment discontinuation. The patient was made cadaver kidney transplants 10 months after treatment. The patient who has still HCVRNA negativity is being followed no problem. The results of our patient who received haemodialysis, were candidates for kidney transplantation is promising for patients with chronic hepatitis C who were stopped treatment prematurely because of side effects.

P-0151

Acute hepatitis and reactivation of hepatitis C virus in patients under immunosuppressive therapy**Hae Lim Lee, Si Hyun Bae, Jeong Won Jang, Jong Young Choi, Seung Kew Yoon**

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Introduction: Reactivation of hepatitis C virus(HCV) has been known as unusual event and only a few cases with severe complications have been reported under immunosuppressive therapy. This retrospective study was aim to evaluate the effect of the therapies on clinical course of HCV infected patients.

Methods: A total of 889 patients screened for the presence of anti-HCV antibody and underwent systemic chemotherapy, steroid or immunosuppressive agents were reviewed from January 1, 2008 to March 1, 2015. 751 patients were excluded because of as follows; patients did not check HCV RNA titers, had history of treatment for chronic hepatitis C(CHC) and other liver diseases except CHC.

Results: Among the 138 patients, 64 (46 %) showed flare of ALT of all cause. There was significant difference between the two groups in disease categories divided as flare of ALT or not. 27 (42 %) of the 64 patients had hematological tumors. In subgroup analysis of patients

with detectable HCV RNA, similar tendency was shown. Levels of HCV RNA were checked in 37 patients before and after the therapies. HCV RNA reactivation was noted in 10 (27 %) patients and flare of ALT associated with HCV RNA reactivation was noted in 7 (19 %) patients. There was no significant factor for the prediction of HCV RNA reactivation. There was no severe hepatitis accompanied by jaundice or hepatic decompensation.

Conclusion: HCV RNA reactivation can be induced during the treatments. However, there was no predictable factor. Patients with HCV infection can be generally treated without concern regarding further deterioration of their condition.

P-0152

Acute hepatitis c infection in human immunodeficiency virus (HIV) infected case**Nurbanu Sezak, Kaptan Figen, Bahar Ormen, Nesrin Turker, Serap Ural, Sibel El, Tuna Demirdal**

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Introduction: HIV-HCV co-infection is not rare because of their common transmission routes. Acute HCV infection is increasing among HIV infected MSM population. HIV-infected case with acute HCV infection is presented here.

Case: A 39 years-old, single man, was admitted to our clinic with his HIV-positive test result, 5 years ago. He had a history of bisexual intercourse. Laboratory examination revealed that HIV-RNA: 7×10^4 c/ml, HBsAg: (–), Anti-HBc: (+), Anti-Hbs: (+), Anti-HAV-IgG: (+), Anti-HCV: (–). While viral suppression and immune-reconstruction was achieved with antiretroviral treatment, fenofibrate was given due to hypertriglyceridemia despite of diet and exercise program. We noticed an increase in transaminases while lipid levels were falling. Because of that anti-HCV and HCV-RNA tests were repeated. According to test results acute HCV (genotype 3) infection was diagnosed. Fenofibrate treatment was discontinued and pegylated interferon alfa-2a (180 mcg) and ribavirin treatment was started with antiretroviral treatment (tenofovir/emtricitabine + lopinovir/ritonavir). HCV-RNA was negative at the forth week of treatment. Treatment for HCV infection continued for 24 weeks. HCV-RNA was negative at the end of treatment and sustained viral response was achieved.

Conclusion: Spontaneous healing rate is about 20 % for HIV-HCV co-infection. Treatment should be started 4 weeks after the diagnosis of acute HCV infection if there is a decline of less than 2 log HCV-RNA in HIV-infected patients. Pegylated interferon and ribavirin should be used for 24 weeks. As a result of this study, HIV-infected patients under anti retroviral treatment should be monitorized for acute HCV infection if they have dubious sexual behaviors.

P-0153

Acute Hepatitis C Infection: A case report**Nesrin Turker, Bahar Ormen, Serap Ural, Selin Ozdemir, Ilknur Vardar, Atakan Nemli, Tuna Demirdal**

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Introduction: Acute Hepatitis C virus (HCV) infection is defined as acute hepatitis which is presenting within 6 months of the exposure. The vast majority of cases of occupational HCV transmission have involved percutaneous exposures. We aimed to present an acute HCV infection case following an occupational needle stick injury.

Case: A 38 years old female nurse was referred to infectious diseases department who had a history of percutaneous needle stick injury while caring a patient 3 weeks ago and had no other risk factors for HCV infection. Hepatitis serology of source patient was unknown. She was vaccinated for hepatitis B virus and anti HCV was documented as negative before that occupational exposure. Hepatitis serology was studied and anti HCV was found positive. ALT and AST levels were 726 and 622 IU/L respectively. HCV PCR was positive (152 577 IU/mL, genotype 1b). She was diagnosed as acute HCV infection. The patient was monitored and treatment was delayed for 12 weeks to allow spontaneous clearance. HCV-RNA was still positive after 12 weeks and pegylated interferon alpha 2b (120 mcg/week) treatment was initiated. At 4th week of therapy rapid virologic response was achieved and this response was maintained throughout her treatment course for 24 weeks. After completion of therapy sustained virologic response was achieved.

Conclusion: Most of patients who acquire HCV, including occupational acquisition, do not have obvious clinical symptoms associated with seroconversion. Preventive strategies and policies should be established and health care workers should be trained for procedures following occupational exposures.

P-0154

Is There Any Impact Of Thyroid Dysfunction On EVR In Patients With Acute HCV Infection?

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Introduction: There is data gained by controlled randomized clinical trials that indicates thyroid dysfunction in up to %20 of patients undergoing interferon-based therapies (IFN-BAT) for chronic HCV infection in current literature. Data regarding the frequency and severity of these changes due to IFN-BAT are still scant in acute HCV infection. We want to evaluate whether there is any effect of this alteration if exist in this population.

Methods: Data of 48 patients treated for acute HCV infection with pegylated interferon (PEG-IFN) with or without ribavirin(RBV) (Group A) in Kocaeli University Gastroenterology Department were compared with those of 210 patients with chronic HCV infection who have taken PEG-IFN/RBV treatment (group B) at same institute. Thyroid dysfunction was estimated by serum TSH levels. TSH levels above or below the normal range were classified as abnormal.

Results: After starting IFN-BAT, 27/210 (12.8 %) patients developed abnormal TSH values in group B. In group A, 7/48 (14.6 %) patients experienced abnormal TSH values which was not different in comparison to those in group B patients. Regarding virologic response, 48.8 % (20/41) of patients with normal TSH values achieved an early virologic response (EVR) in treatment week 12 in contrast 57.1 % (4/7) in patients who experienced alternated TSH levels. There is no patient in both groups discontinued therapy because of thyroid dysfunction.

Conclusion: Thyroid dysfunction triggered by PEG-IFN seems not to be associated with a lower EVR in acute hepatitis C.

P-0155

Fib5: A Novel Test Differentiating between Non-significant and Significant Fibrosis in CHC patients

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Introduction/Aim: A simple noninvasive score (Fib-5) using five routine blood tests; namely ALT, AST, ALP, Albumin and Platelets; allows the detection of significant hepatic fibrosis in patients with chronic hepatitis C (CHC). The FIB-4 index is another test that can identify the group of patients. This study assesses the performance of Fib-5 and Fib4 tests performed in the same day regarding their ability to differentiate between non-significant (F0-F1) from significant fibrosis (F2-F4).

Methods: A total of 604 patients with biopsy-proven CHC were included. All biopsies were scored using the METAVIR system. Both scores (Fib-5 and Fib-4) were measured and the performance characteristics were calculated using Area Under the ROC curve.

Results: Out of the 604 patients, 391 (64.7 %) had F0-2 and 213 (35.3 %) had F3-4, 18 (3 %) of them had F4. The Area Under the ROC curve for Fib-5 and Fib-4 in the detection of significant fibrosis were 0.818 and 0.713 respectively. The specificity and the positive predictive value (PPV) of Fib-5 at more than or equal to 7.50 for the differentiation between F0-F1 and F2-F4 were 94.4 and 85.7 % respectively, and those of Fib-4 at less than or equal to 1.45 were 54.9 and 55.7 % respectively.

Conclusion: The Fib-5 score at the new cut-off (7.50) is more specific than the FIB-4 index for the differentiation between non-significant and significant fibrosis in patients with CHC.

P-0156

Long-term clinical outcome of HCV decompensated cirrhotic patients after antiviral treatment

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Objective: To investigate the efficacy and safety of antiviral treatment and the effects of virological response on long-term prognosis in HCV decompensated cirrhotic patients.

Methods: From August 2008 to August 2013, 66 consecutive, IFN-naïve HCV decompensated cirrhotic patients treated with PEGIFN a-2b/PEGIFN a-2a once weekly or IFN-a-2b every other day, plus ribavirin for 48–72 weeks, with a low accelerating dosage regimen

were included in this prospective study. Patients were routinely monitored for adverse drug reaction and virological response and its influence on long-term prognosis.

Results: Forty-nine patients were HCV RNA negative at end of treatment (ETVR), thirty of them achieved SVR (45.5 %) and 19 of them were relapse (28.8 %), and remaining 17 (25.7 %) patients were NVR. 65.9 and 34.1 % patients with genotype 1 achieved ETVR and SVR; and 90.9 and 68.2 % patients with genotype 2 achieved ETVR and SVR respectively. Early virological response (EVR) as the positive and negative predictive value of ETVR were 95.7 and 75 %; and EVR as the positive and negative predictive value of SVR were 65.2 and 100 % respectively. Patients achieved ETVR were associated with improved liver function including TBIL, ALT, ALB, PTA and Child-Pugh score, and reduced risk of hepatic decompensation and HCC, and improved survival. Twelve (18.2 %) out of these patients experienced serious adverse events and 2 died.

Conclusion: Antiviral treatment for HCV decompensated cirrhotic patients with interferon in a low accelerating dosage regimen in combined with ribavirin is feasible. Patients achieved ETVR after IFN-based therapy had a significant improved long-term prognosis.

P-0157

Progression of liver fibrosis in HCV genotype 3a is associated with modulation in let-7 miRNA family

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Chronic HCV infection is major cause of liver fibrosis and end stage liver disease. There is limited data on systematic miRNA based biomarker study on hepatic fibrogenesis in HCV genotype 3a. We profiled circulating miRNAs in plasma of HCV G3a infected patients with different stages of hepatic fibrosis. 47 subjects with histologically proven chronic hepatitis C were categorized based on stage of hepatic fibrosis: F0–1 (n = 32), F3–4 (n = 15) and compared with healthy controls (n = 28). Differentially expressed miRNAs in plasma of 4 subjects each of healthy controls, F0–1 and F3–4 were studied and subsequent validation by stem-loop RT-PCR. miRNA target gene prediction was done by multiMiR analysis. Of the total 185 miRNAs screened in the panel, 31 miRNAs were commonly identified in all the groups. Three miRNAs were significantly down regulated ($p < 0.01$, fold change < -3) when F0–1 were compared to F3–4. ΔCt for most significantly downregulated miRs in F0,1 vs F3,4 were: let-7a (-0.1671 ± 0.6263 vs -1.465 ± 0.8206 , $p < 0.0001$), let-7c (-3.345 ± 0.8334 vs -4.384 ± 0.8114 , $p = 0.0012$) and let-7f (-2.515 ± 1.105 vs -3.970 ± 0.9538 , $p = 0.0006$). The AUROC for all the validated miRNAs in fibrosis vs healthy subjects were AUROC > 0.9 , $p < 0.01$, while in F0,1 vs F3,4 AUROC > 0.8 , $p < 0.01$). ΔCt cut-off of let-7a at -1 was most discriminatory miRNA between HCV and healthy subjects with 92 % sensitivity and 73 % specificity. All the validated miRs correlated negatively with the stage of fibrosis ($r^2 > -0.8$; $p < 0.0001$). In-silico analysis revealed extracellular matrix interaction pathway as the most enriched pathways, $p = 1.94E-16$. Our findings indicate significance of let-7 family miRs in HCV mediated liver fibrosis progression.

P-0158

Association of SVR and All-cause Mortality Post INF-based Therapy for CHC in a US Community Setting

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Background: Previous tertiary care center studies have shown that sustained virological response (SVR) to interferon-based therapy for HCV is associated with a reduction of all-cause mortality. This study assesses whether this finding is applicable in a community-based health care setting and examines for differences per cirrhosis status. **Methods:** This is a retrospective cohort study at Kaiser Permanente, Southern California (KPSC), a community-based integrated health care system including 3.5 million members, with mortality data from the Vital Statistics of California Report. Inclusion criteria: a diagnosis code and/or positive HCV RNA test (index date) 1/1/02–12/31/13; ≥ 18 years at index date, ≥ 12 months continuous membership before and after index date. Exclusion criteria: HCV diagnosis after 1/1/13, liver transplant or HCC on or before index date. SVR was determined for patients treated for HCV with interferon-based therapy and stratified for cirrhosis versus non-cirrhosis.

Results: Total chronic HCV cohort was 24,968 (42 %, had cirrhosis). Overall mortality during the study period was 18.5 % (4608/24,968): 33.2 % (3470/10,449) among cirrhotics vs 7.8 % (1138/14,519) among non-cirrhotics. For patients treated for HCV, 45.1 % (2348/5203) achieved SVR; 5.4 % mortality (127/2348) for those who achieved SVR vs 18.9 % (540/2855) for those without SVR.

Conclusions: An approximate 3-fold reduction in all-cause mortality is seen in treated HCV patients who achieve SVR compared to those without SVR. Treated cirrhotics who achieve SVR have a >3 -fold reduction in mortality versus treated cirrhotics who do not. Hence new, more potent, HCV treatment regimens with higher SVR can potentially reduce future HCV-related mortality significantly.

Table 1. Mortality in HCV patients treated with interferon-based therapy: Cirrhosis: N=2603; No Cirrhosis: N=2600

	HCV, Received Treatment	Cirrhosis/SVR	Cirrhosis/No SVR	No Cirrhosis/SVR	No Cirrhosis/No SVR
N (%)	5203	989 (38%)	1614 (62%)	1359 (52.3%)	1241 (47.7%)
Mortality, N (%)	667 (12.8%)	77 (7.8%)	415 (25.7%)	50 (3.7%)	125 (10.1%)

P-0159

Age and female are the risk factors for HCC even in chronic hepatitis C patients with SVR

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Background and Aims: Recently, majority of chronic hepatitis C patients could achieve the sustained virological response (SVR) by highly effective direct-acting antivirals. The *Wisteria floribunda* agglutinin-positive human Mac-2-binding protein (WFA⁺-M2BP) is a novel, non-invasive serum liver fibrosis glycomarker. Consistently elevated post-treatment serum WFA⁺-M2BP levels have been reported to be associated with the development of hepatocellular carcinoma (HCC). However, the change of the serum WFA⁺-M2BP level after SVR is unknown. This retrospective study was done to evaluate the factors that affect post-treatment serum WFA⁺-M2BP levels by chronic hepatitis C patients.

Methods: Serum WFA⁺-M2BP levels 1 year after the end of treatment were analyzed in 110 chronic hepatitis C patients who achieved SVR.

Results: The normalization (<1.0 COI) of serum WFA⁺-M2BP was shown in 42 (38.2 %) patients (Group A), while the level is still abnormally high (>1.0 COI) in 68 (61.8 %) patients (Group B). The pretreatment serum albumin levels and platelet count of the Group A patients were significantly higher than Group B, while the pretreatment FIB-4 index of the Group B was significantly higher than Group A. Furthermore, the pretreatment median age of the Group B was significantly higher than Group A (64 vs. 54 years old, respectively, $P = 0.012$). Multivariate analysis extracted age (>60 years old) and female as risk factors of non-normalization of serum WFA⁺-M2BP level after SVR.

Conclusions: Our results suggest that elderly patients and female still have the risk of development of HCC due to unresolved liver fibrosis even after SVR.

P-0160

Efficacy and safety of dual oral therapy with DCV and ASV after curative RFA of HCV-related HCC

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We administered daclatasvir and asunaprevir for patients with hepatocellular carcinoma (HCC) treated by radiofrequency ablation (RFA) and evaluated short term efficacy and safety of this treatment. Patient Characteristics ($n = 27$) were as follows: 17 male and 10 female; 73 years old (54–82); chronic hepatitis were 8 cases and liver cirrhosis were 19 cases; AST 53(31–156) IU/l, ALT 49 (20–205) IU/l, platelet count 76,000 (39,000–360,000) / μ l, AFP 16 (4.0–99) ng/ml and HCV-RNA 6.1 (4.4–7.2) log IU/ml. Amino acid substitutions analyzed using PCR-Invader were as follows: Y93H mutation type were 2 cases, Y93 wild type were 20 cases, and undeterminable type was 1 case; L31 M/V mutation type was no case, L31 wild type were 22 cases, and undeterminable type was 1 case. Amino acid substitutions analyzed using direct sequence analysis were as follows: Y93 mutation type was 1 case, Y93 wild type were 3 cases, and Y93 mixed type was no case; L31 mutation type was no case, L31 wild type were 3 cases, and L31 mixed type was 1 case. Antiviral effects were as follows: RVR was 1 case; EVR were 11 cases; SVR 4 were 2 cases; SVR 12 were 5 cases; SVR 24 were 2 cases; breakthrough was 1 case. Stopped administering these medicines were 6 cases (4 of 6 cases were HCC recurrence and 2 of 6 cases were fever). Dual oral therapy of daclatasvir and asunaprevir was safe and efficacious in patients after curative treatment of HCC.

P-0161

Direct acting antiviral agents can be safely used for hepatitis C with hepatocellular carcinoma

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Purpose: Since September 2014, the first direct acting antiviral agents (DAAs), daclatasvir (DCV) and asunaprevir (ASV) combination therapy have been introduced for the patients with HCV positive patients. Here we investigated the outcome of difference between the patients in the presence or absence of the history of HCC in DAAs therapy.

Method: Sixty five patients with chronic hepatitis and 37 compensated cirrhotic patients with HCV genotype 1 were treated by DCV 60 mg/day and ASV 200 mg/day for 12 weeks. Twenty four patients having past history of HCC were compared with those without the history of HCC.

Results: Although gender, age, platelet count, liver function test and the titer of HCV RNA were not different in the two groups, body mass index (BMI) was significantly lower in HCC-treated group (24.4 ± 4.4 vs. 22.3 ± 2.7). In terms of therapeutic effect, rapid virological response (RVR) was comparable between them (74.4 vs. 79.2 %). As for the side effects, no differences in liver dysfunction and subjective symptoms between the two groups were observed. Noteworthy, 4 patients recurred HCC during the DAAs therapy were all accompanied with LC. They were all treated by transcatheter arterial chemo embolization and percutaneous radiofrequency ablation and achieved complete remission.

Conclusion: DAAs therapy can be indicated for the patients with past history of HCC without any differences of the effect and adverse events compared with those of no history of HCC. DAAs therapy can be tolerable and positively recommended for the patients with past history of HCC.

P-0162

Genetic variants near HLA gene were associated with the risk for HCC among HCV infected patients

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Background: This study aimed to discover genomic variations associated with HCC risk through the genome-wide association study and imputation analysis.

Methods: There were 472 HCC cases and 806 unaffected controls. All study subjects were seropositive for anti-HCV and seronegative for HBsAg. Human genomic DNA was extracted from blood sample to perform genotyping by AxiomTM Genome-Wide CHB Array. Imputation algorithm was applied to get whole genome variations in patients based on genotyping data from single nucleotide polymorphism (SNP) arrays. The reference genomes of imputation were Han Chinese population in 1000 genome project. The logistic regression was used to evaluate association between HCC and genotype based on four genetic models (allelic, dominant, additive and recessive). The imputed SNPs significantly associated with HCC were further validated by TaqMan Assay.

Results: A total of 36,175,343 SNPs were obtained after imputation. There were seven nonsynonymous SNPs found to be significantly associated with HCC under different genetic models. The adjusted odds ratios (ORs) of the imputed SNPs associated with risks for HCC ranged from 0.67–2.26 ($p < 0.05$). Interestingly, the seven nonsynonymous SNPs clustered on the human leukocyte antigen (HLA) complex region. There were three SNPs still associated with HCC risk via TaqMan genotyping assay in dominant and recessive models. The adjusted ORs for two SNPs in dominant models were 1.61 (1.10–2.36) and 0.63 (0.44–0.91). The SNP in recessive model showed 2.03 folds risk (95 % CI 1.33–3.10) for HCC.

Conclusions: The host variants were associated with risk for HCC among patients with hepatitis C virus infection.

P-0163

Influence on hepatitis C virus eradication on hepatocellular carcinogenesis

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The major cause of hepatocellular carcinoma (HCC) in Japan is chronic HCV infection and accounts for about 60 %. Therefore sustained virologic response (SVR) is essential to prevent HCCs.

Subjects: We compared the two groups (A, B) of patients in this study: 12 patients (group A) who achieved SVR before the development of HCCs and 5 patients (group B) who achieved SVR after the curative treatment of initial HCCs.

Methods: We reviewed various background factors and the time to occurrence or recurrence of HCCs after each achievement of SVR. And we calculated occurrence or recurrence free survival. [Results] Group A: Median time to HCC occurrence after SVR was 264 weeks. The average liver stiffness (LS) by FibroScan was 18 kPa at the beginning of interferon therapy and 12 kPa at HCC occurrence respectively. After the curative treatment, four patients had relapsed ectopically. Median time to HCC recurrence and recurrence-free survival was 224 and 271 weeks respectively. Group B: The average period from HCC curative treatment to the initiation of interferon therapy was 30 weeks. The average LS was 13.7 kPa at the beginning of interferon therapy and 10.5 kPa after SVR. After SVR, two patients had relapsed ectopically. The median time to HCC recurrence and recurrence-free survival was 232 and 223 weeks respectively.

Conclusion: If HCV are eradicated irrespective of before or after occurrence of initial HCCs, the occurrence or recurrence rate of HCCs

may decrease and the prognosis may improve. The antiviral therapy and surveillance after SVR should be strongly recommended.

P-0164

Risk factor for development of HCC in patients who are eradicated hepatitis C virus

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Background: HCV genotypes and single nucleotide polymorphism of IFNL3 are strongly associated with response to IFN therapy, but the relationship between HCC is unknown. The aim of this study is to investigate the incident rate of HCC and clarified the risk factors including HCV genotypes and IFNL3 polymorphism of HCC in SVR patients.

Method: We investigated the incidence of HCC in 639 patients who achieved SVR by IFN based therapy. The median period of follow-up was 7.8 years.

Result: Six patients were developed HCC after SVR. The incidence of HCC was 0.43 % at 2 years, and 1.4 % at 5 years. The median period of HCC development was 2.8 years (range 1.3–5.6). IFNL3 polymorphism was major allele in all patients who developed HCC. Cox proportional hazards regression analyses showed that lower platelet count was independently risk factor for development of HCC (hazard ratio 8.92; $P = 0.050$). The other factors such as HCV genotype and IFNL3 polymorphisms were not significant factors for development of HCC. The cumulative rates of incidence of HCC in patients with the lower platelet count ($<15 \times 10^4/\text{mm}^3$) were 1.12 % at 5 years, and those with in the higher platelet ($>15 \times 10^4/\text{mm}^3$) were 2.3 % at 5 years. There was a significant difference in carcinogenesis rate in the two groups ($P = 0.017$).

Conclusion: HCV genotypes and SNP of IFNL3 were not associated with development of HCC but the lower platelet count would be one of important features for HCC development in patients after SVR by IFN based therapy.

P-0165

Clinical model for predicting the post-SVR of HCC in CHC patients: A case control study

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Background: A clinical model for predicting the occurrence of hepatocellular carcinoma in sustained virologic response-achieving (post-SVR HCC) chronic hepatitis C (CHC) patients is lacking.

Methods: We performed a case control study using a clinical database to research the risk factors for post-SVR HCC. A predictive model based on the risk factors was established, and the area under the receiver operating characteristic curve (AUC) was calculated.

Results: In the multivariate model, the initial diagnosis of compensated cirrhosis and post-SVR albumin reductions of 1 g/L were

associated with 21.7 (95 % CI 4.2–112.3, $p < 0.001$) and 1.3-fold (95 % CI 1.1–1.7, $p = 0.004$) increases in the risk of the occurrence of post-SVR HCC, respectively. A predictive model based on the initial diagnosis as compensated cirrhosis (yes, 1; no, 0) and post-SVR albumin less than or equal to 36.0 g/L (yes, 1; not, 0) was able to forecast the occurrence of post-SVR HCC with a cutoff value of >0 , an AUC of 0.880, a sensitivity of 0.833, a specificity of 0.896, and a negative predictive value of 0.956.

Conclusions: Regarding risk factors, an initial diagnosis of compensated cirrhosis combined with a post-SVR albumin value less than or equal to 36.0 g/L can predict the occurrence of post-SVR HCC in CHC patients.

P-0166

Direct Medical cost for Hepatitis C virus infection in South Korea, 2009–2013

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Background/Aim: The study sought to elucidate the nationwide number of insured patients due to chronic hepatitis C from National Health Insurance Service, and the annual direct medical cost per patient in South Korea.

Methods: The Health Insurance Review and Assessment Service (HIRA) claims data on chronic hepatitis C virus (HCV) infection identified with ICD 10 code of B18.2 as the primary or additional diagnoses were obtained from 2009 through 2013. The number of patients with demographical characteristics, and medical costs were analysed.

Results: The number of insured HCV patients was approximately 68,000 per year. The total medical cost of both out-patients and in-patients was on average 62.2 million USD per year and 910 USD per patient per year. For the cost, antiviral drug cost was responsible for 36 %, followed by the examination fee, except ultrasonography, which was 25.6 %, and then hospital fee, doctor's fee, and operation fee as 9.0, 8.9, and 7.9 %, respectively. Among those who received peginterferon-alpha-2a and ribavirin, the annual cost was 3970 USD per patient. The first-year and the second-year cost for patients who developed cirrhosis were 1970 USD and 1710 USD, respectively. Those for hepatocellular carcinoma (HCC) were 10100 USD and 6700 USD, respectively. Those for liver transplantation (LT) were 76770 USD and 9670 USD, respectively.

Conclusion: Medical costs increased remarkably from chronic hepatitis to liver cirrhosis, to HCC and LT. Every effort to intervene disease progression should be exerted to reduce the HCV disease burden.

P-0167

Disease Burden of Chronic Hepatitis C Virus (HCV) Infection in Japan

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Background: HCV infection is a leading cause of hepatocellular carcinoma (HCC) in Japan. New antiviral treatments achieve higher sustained viral response rates (SVR) than the current standard of care. Implementation of new treatments requires epidemiological data and modeling to assess the potential impact of improved treatment strategies.

Methods: Disease progression used age-and gender-defined cohorts to track HCV incidence, prevalence, morbidity and mortality. The relative impacts of two scenarios on HCV-related disease burden were considered: (1) No antiviral treatment during 1992–2030) Annually treat up to 26,860 patients (2012–2030) with SVR increased to 95 % in 2016.

Results: If the current treatment paradigm continues (26,860 treated annually), chronic infections decline to 271,000 in 2030 as compared to 1,014,000 in 2014 (75 % decrease), largely due to mortality. The number of incident HCC cases is projected at 5840 in 2030, a decrease of 50 % as compared to 2014 (23 030 incident HCC). Under scenario 1, chronic infections in 2014 are estimated at 1,223,000 (20 % increase from base). Incident HCC cases in 2014 and 2030 were estimated at 25,070 and 12,050, respectively (10 % and 105 % increase from base). Under scenario 2, viremic cases in 2030 were estimated at 206,700 (25 % decrease from base). Incident HCC cases in 2014 were estimated at 3340 in 2030 (45 % decrease from base).

Conclusions: Base case results show a substantial burden of HCV-related liver disease, while scenario 1 demonstrates that disease burden would be greater if treatment were not implemented in 1992. Treatments with greater efficacy can reduce disease burden.

P-0168

Target of rapamycin and autophagy in chronic hepatitis C infection: relation to disease activity

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Background: In chronic hepatitis C (CHC) infection, liver disease progression results from viral persistence. Deregulation of target of rapamycin (TOR) signaling and autophagy is detected in viral infections and cancer. We studied the role of TOR and autophagy in the progression of CHC infection.

Methods: 54 patients infected with HCV, [27 with CHC; 13 with cirrhosis and 14 with hepatocellular carcinoma (HCC)]; and 15 healthy subjects were included. Quantification of serum TOR was determined using enzyme linked immunosorbent assay. Tissue samples were immune-stained using TOR and autophagy protein 5 (Atg5) polyclonal antibodies. Expression of TOR and Atg5 was scored semi-quantitatively.

Results: Expression and serum TOR were higher in HCC patients compared to patients without HCC ($P < 0.05$). Serum and expression of TOR were positively correlated to inflammation, fibrosis, steatosis and tumor characteristics (Alpha-fetoprotein, maximum diameter, tumor grade and CLIP stage) ($P < 0.05$). Expression of Atg5 was inversely correlated with inflammation, fibrosis and tumor characteristics ($P < 0.05$). Atg5 showed significant inverse correlation with serum and intrahepatic expression of TOR ($P < 0.05$). Serum TOR showed high sensitivity and specificity in discriminating patients with and without HCC at a cut-off value of 4.55 ng/ml [AURC = 0.970].

Conclusions: Activation of TOR plays an important role in HCV related liver disease progression possibly through autophagy suppression and they represent a potential therapeutic target. Serum TOR is a potential seromarker in discriminating HCV infected patients with and without HCC with high sensitivity and specificity.

P-0169

Assessing the economic impact of new direct-acting antivirals for chronic hepatitis C in Japan

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Background: Novel direct-acting antiviral (DAA) regimens with high rates of sustained virologic response (SVR) and improved tolerability have revolutionised the treatment of chronic hepatitis C virus (HCV). The study objective was to assess the economic value and budget impact (BI) of treatment strategies involving the DAA regimens daclatasvir + asunaprevir (DUAL) and sofosbuvir/ledipasvir (Harvoni) for patients with HCV genotype 1b without NS5A resistance-associated polymorphisms (RAPs) in Japan.

Methods: A published HCV Markov model was used to perform a cost-effectiveness analysis (CEA) of DUAL (24 weeks: ¥2,645,568) versus Harvoni (12 weeks: ¥6,734,388). SVRs (99.3 and 100 %, respectively) were derived from a matching-adjusted indirect comparison. A de novo BI model was developed to predict the 3-year cost implications of alternative treatment pathways (initiating with DUAL or Harvoni and re-treating failures with the opposite regimen), applying assumed uptake rates. Model inputs, transition rates and discounting (2 %) were specific to the Japanese setting.

Results: CEA results demonstrated minimal difference in benefit (additional 0.022 QALYs and 0.002 life-years with Harvoni); however, DUAL was estimated to have a significantly lower total cost than Harvoni (difference of ¥4,085,453). The BI analysis demonstrated that increasing the proportion of patients initially treated with Harvoni from 0 to 100 % led to total costs increasing from ¥242 to ¥586 billion; i.e. first-line treatment with DUAL is expected to save ¥344 billion.

Conclusions: Based on results from conventional CEA and BI analyses, first-line treatment with DUAL is expected to be highly cost-saving compared to Harvoni in Japan for HCV patients without NS5A RAPs.

P-0170

Disease burden of chronic hepatitis C virus (HCV) infection in Taiwan

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Aim: A modeling approach was used to examine HCV-related disease progression and evaluate scenarios required to control or eliminate HCV. **Method:** The infected population and disease progression were modeled using age- and gender-defined cohorts to forecast HCV incidence, prevalence, hepatic complications and mortality. Baseline assumptions and transition probabilities were extracted from the literature. The impacts of two scenarios of increased sustained virological response (SVR), treatment, and diagnosis were compared to the baseline scenario. **Results:** Under the baseline scenario, 529,000 individuals were chronically infected in 2014. By 2030, the prevalence was projected to decrease 42 %, however advanced stage liver disease and mortality would increase by 21–27 %. Under a disease control scenario, in 2014, SVR increased to 99 % (among 15–84 years, ≥F0) with the annual number treated static at 8600. Compared to the baseline in 2030, there was a 5 % reduction in prevalent cases and a 7–9 % reduction in advanced stage liver disease and death. Under an elimination scenario, the same increases in SVR along with a 3-fold increase in annual number diagnosed and treated resulted in a 79–89 % reduction of advanced stage liver disease and mortality and a 91 % reduction in viremic prevalence compared to the baseline in 2030. See table for full results.

Conclusion: A scenario that considered increases in SVR, number treated, and number diagnosed had a significantly greater impact than a scenario which considered increased SVR alone. The projected impact of the scenarios will facilitate disease forecasting, resource planning, and strategies for HCV management.

Table: Modeled HCV burden, 2014 and 2030

	2014	2030		
		Base*	Increase in SVR only	Increase in SVR and Tx
New Cases (2014-2030) (10 ⁵)	2.4	2.4	2.4	2.4
Viremic Cases (10 ⁵)	528.8	322.6	305.0	27.8
Compensated LC (10 ³)	66.5	80.6	74.6	9.2
Decompensated LC (10 ³)	7.5	9.5	8.6	1.3
Liver Cancer (10 ³)	3.5	4.3	4.0	0.5
Liver-related Deaths (10 ³)	4.1	5.2	4.8	1.1

*Base: current rates of new cases and treatment with Peg

P-0171

Gadoxetic acid enhanced MRI predicts hyperbilirubinemia of simeprevir in patients with HCV

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Background and aims: Simeprevir, a hepatitis C virus NS3/4A protease inhibitor, is an inhibitor of bilirubin transporters, including organic anion-transporting polypeptides (OATPs). Hyperbilirubinemia was often occurred as an adverse event during triple therapy, simeprevir plus pegylated interferon/ribavirin. We aimed to clarify the relationship between bilirubin increase and hepatic enhancement with gadoxetic acid, also a substrate of OATPs, enhanced MR imaging.

Methods: A total of chronic hepatitis C 11 patients, treated with simeprevir with pegylated interferon plus ribavirin, were calculated the contrast enhancement index (CEI), as an index of liver

parenchymal enhancement on the hepatobiliary phase before the treatment. We measured the plasma trough levels (Ctough) of simeprevir seven days after the administration.

Results: Hyperbilirubinemia of greater than 1.5 mg/dl were observed in 6 (55 %) patients during the therapy. Ctough were significantly higher in group with hyperbilirubinemia than in group without hyperbilirubinemia ($P = 0.017$). CEI levels were significantly lower in hyperbilirubinemia group than in non-hyperbilirubinemia group ($P = 0.007$). We found a remarkably strong inverse relationship between levels of CEIs and Ctough ($R = -0.911$). Serum albumin and FIB-4 index were related with Ctough ($R = -0.657$, $R = 0.804$, respectively). A significant partial correlation coefficient ($R = 0.872$, $P < 0.01$), which involves studying the linear relationship between CEIs and Ctough after excluding the effect of serum albumin and FIB-4 index was found.

Conclusion: Hepatic parenchymal enhancement with gadoxetic acid could be related to the plasma concentration of simeprevir and thereby could predict the onset of hyperbilirubinemia.

P-0172

Abnormal iron metabolism in patients with chronic hepatitis C

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Hepatic iron accumulation, as well as oxidative DNA damage, is significantly increased in CHC livers. However, the precise mechanism of iron accumulation in the CHC liver remains unclear. In this study, 24 cases of CHC and 9 cases of control were enrolled. An oral iron absorption test (OIAT) was used, in which 100 mg of sodium ferrous citrate was administered to each individual. The OIAT showed that absorption of iron from the gastrointestinal (GI) tract was increased significantly in CHC patients, compared to control subjects. Serum hepcidin concentration was significantly elevated in patients with CHC. As well as, messenger RNA (mRNA) level of Ferroportin-1 (FP1) in the duodenum was significantly elevated in patients with CHC. The OIAT results were correlated hepcidin and FP1 mRNA levels. Then, to demonstrate that FP1 mRNA levels increase by hepcidin, we cultured Caco-2/TC7 cell monolayers cultured in transwells with or without hepcidin. The FP1 mRNA levels were significantly elevated in the Caco-2/TC7 cell monolayers cultured with hepcidin. In CHC patients, iron absorption from the GI tract increased through up-regulation of FP1 by serum hepcidin elevation.

P-0173

Aspartate transaminase to platelet ratio index (APRI) in HCV and Schistosomiasis coinfection

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To assess the diagnostic accuracy, of aminotransferase to platelet ratio index (APRI) alone and with antischistosomal antibody (Ab) in patients with hepatitis C virus (HCV) and schistosomiasis coinfection. **Methods:** A 383 patients, undergone liver biopsy between January 2006 to April 2014 in Qatar were selected. HCV RNA titer and antischistosomal antibody titer were assessed.

Results: Median age of patients was 46 years. About 7.1 % had no fibrosis, whereas 30.4, 37.5, 20.4, and 4.6 % had fibrosis of stage I, II, III, and IV respectively. In bivariate analysis, APRI score, levels of AST, platelets count and age of patient showed statistically significant association with liver fibrosis ($p < 0.0001$); whereas antischistosomal antibody titer ($p = 0.52$) and HCV RNA titer ($p = 0.79$) failed to show a significant association. The respective AUC values for no fibrosis, significant fibrosis, severe fibrosis and cirrhosis of APRI score were 63, 73.2, 81.1 and 88.9 % respectively. This showed good sensitivity and specificity of APRI alone for grading of liver fibrosis. But the inclusion of anti-Schistosoma antibody did not improve the prediction of fibrosis stage.

Conclusion: The study results suggest that noninvasive biochemical markers like APRI are sensitive and specific in diagnosing the degree of fibrosis and cirrhosis in patients with coinfection of HCV and schistosomiasis as compared to biopsy. The addition of antischistosomal Ab to APRI did not improve sensitivity for predicting the degree of cirrhosis.

P-0174

Cost-effectiveness of response-guided therapy with triple DAAs in non-cirrhotic CHC GT1b

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Background and Aim: Addition of an NS3 protease inhibitor to dual NS5A-NS5B nucleoside analogs can enhance antiviral efficacy and shorten treatment duration to 3-weeks in non-cirrhotic Chinese with chronic hepatitis C (CHC) GT1b subjects with rapid virologic response, with plasma HCV RNA < 500 IU/ml by 48 h. We evaluate the cost-effectiveness of a 3-weeks treatment using Harvoni® (ledipasvir/sofosbuvir) and asunaprevir (ASV), compared with standard-of-care 8–12 weeks treatment using Harvoni®.

Methods: A decision analytic Markov model with a lifetime horizon and a third party perspective was developed by treatment history using real-life data. A cycle of 3 week was applied in the first 52 weeks and yearly cycle was applied afterwards. Outcome measures included discounted costs (in 2014 U.S. dollars), quality-adjusted life years (QALYs), and incremental cost-effectiveness ratios (ICERs). One-way sensitivity analysis and probabilistic sensitivity analysis were used to test the robustness of the model.

Results: Compared to 8 or 12-week Harvoni®, 3-week Harvoni®-ASV was cost saving with 0.015 or 0.026 QALY gain and US\$37,454 or US\$63,361 cost reduction per person, respectively. Results were

sensitive to quality of life post sustained virologic response, treatment cost, and treatment effectiveness. At a willingness-to-pay threshold of US\$20,421 per QALY gain (three times GDP per capita in China), 3-week Harvoni®-ASV was cost-effective in 74.9 and 74.4 % treatment-naïve and -experienced patients.

Conclusion: Using response-guided approach, 3-weeks triple therapy with potent triple-DAA regimens containing NS3, NS5A and NS5B inhibitors provided good value for money compared with 8–12 week therapy in non-cirrhotic CHC GT1b subjects.

P-0175

Impact of HCV Treatment on Impoverishment in Western Uttar Pradesh, India

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Aim: To determine any impact of Sofosbuvir based HCV therapy on economic hardship and impoverishment on those starting treatment.

Results: All patients were questioned on their economic status including total family income and source of funding for the therapy at the start of HCV therapy.

Data of 240 patients was analyzed to determine their economic status before and after start of therapy. Family income of 32 Rupees (\$0.5) per person per day was categorized as sustenance income while income above this was referred to as Non-food income (NFx). Total cost of therapy exceeding 40 % of NFx was considered catastrophic (Cat40) if it was an out of pocket payment (OPP). Subjects whose treatment cost exceeded total NFx were considered to have fallen to below poverty line as a consequence of therapy. Treatment expenditure of over 80 % of NFx was labeled as a critical poverty gap indicating being critically near the poverty line.

95.6 % patients made OPP. 6 % were below poverty line at start of therapy, another 8.7 % were within critical poverty gap after start of therapy and 15.3 % fell below poverty line as a consequence of HCV therapy.

Conclusions: Even though the cost of Sofosbuvir based therapy in India is less than \$1200 its impact on impoverishment is serious. Unless OPP is reduced the therapy will continue to have serious social impact.

P-0176

Improved HCV disease awareness and screening compliance following a public media campaign in Israel

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Background: Since most HCV infected patients are asymptomatic, the majority are undiagnosed and untreated. To date, when effective and safe all oral treatments for HCV are available it seems crucial to identify and treat as many HCV patients as possible. In Israel there are about 80,000 HCV infected subjects, the majority are unaware of their disease.

Aim: To increase HCV disease awareness in the Israeli public.

Methods: During 2 months campaign, announcements were published in the Israeli media channels calling patients at-risk to undergo screening for HCV infection and elucidating the importance of timely

treatment. Healthcare professionals provided additional information about the disease by 1–800 hotline. Results were evaluated by analyzing the data from the hotline, by a phone sampling survey before and after the campaign and by calculating the amount of HCV serologic tests consumed following the campaign.

Results: 2400 phone calls were received in the hotline. Data available for 1657 callers and the majority wished to undergo HCV screening. In follow-up calls to 1145 hotline callers, most were either screened for HCV (16 % positive) or referred to specialist. In a survey among 1019 respondents, 55 % were exposed to the campaign and awareness to the disease and treatment options increased. After the campaign, 20 % increase in HCV serologic tests was documented compared to last year. The results demonstrate that a public campaign can increase disease awareness and motivation to screening. Therefore, more patients can be identified and treated. This is crucial nowadays when safe and efficacious therapies are available.

P-0177

Epidemiological characteristics on HIV co-infection with HBV and/or HCV

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Objective: To explore the epidemiological characteristics of HIV and co-infection with HBV and/or HCV in HIV/AIDS patients.

Method: Gathering the epidemiological of 690 cases with HIV/AIDS who had seen doctors in infectious diseases department of The First Affiliated Hospital of Kunming Medical University and The First People's Hospital of Honghe Department during April 2009 to April 2015.

Result: 1. There were 304 cases with HIV and 386 cases with AIDS in all the 690 cases. There were 71 cases with HIV/HBV, 183 cases with HIV/HCV and 26 cases with HIV/HBV/HCV. The rate of HIV/HCV co-infection was higher than the rate of HIV/HBV co-infection. 2. Of all the 690 cases with HIV/AIDS, the ratio male to female was 3:1. The age was from 3 to 82 years old and their average age was 41.05 ± 13.06 years old. Young adults accounted for a large part. They were mainly farmers and migrant workers the number of which was 539. Sexually transmitted way was the main transmitted way in HIV group, HIV/HBV group and HIV/HBV/HCV group, which respectively accounted for 82.93, 84.50 and 69.23 %. Intravenous drug use was the main transmitted way in HIV/HCV group and accounted for 62.84 %. The differences among gender, age, occupation and way of transmission in the four groups were statistically significant (P < 0.05).

P-0178

Prevalence and characteristics of naturally occurring sofosbuvir resistance-associated variants

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Background: Sofosbuvir (SOF), a nucleotide analog pro-drug, targets hepatitis C virus (HCV) NS5B polymerase and shows potential for treating HCV infection, given its high efficacy and good barrier to resistance. However, in addition to the rare resistant-associated variant (RAV) of NS5B S282T, several new potential RAVs of SOF have been reported, especially related to HCV genotype 1b. However, prevalence and characteristics of these RAVs have not been clarified. **Methods:** We analyzed the prevalence of variants in the NS3/NS5A/NS5B regions in 104 patients treated with simeprevir (SMV)-combination therapy, and prevalence of RAVs in patients showing treatment failure was determined by direct- or deep-sequencing methods. Associations between these potential RAVs and clinical factors were also analyzed.

Results: Prevalence of NS5B RAV C316N was high (48.1 %, 50/104), whereas that of NS5B L159F was relatively low (0.96 %, 1/104); however, deep sequencing showed that 30.0 % of patients with C316N also had NS5B RAV L159F. Additionally, there was no significant relationship between the existence of potential NS5B and NS5A or NS3 RAVs. However, the presence of NS5B C316N was significantly associated with an HCV core-aa91 substitution. No significant difference was detected between each RAV and sustained virological response in SMV combination therapy.

Conclusions: We provide the first demonstration of the high prevalence of the potential naturally occurring NS5B RAVs C316N and L159F in Japan. Attention should be paid to these new potential RAVs, especially when conducting SOF-based therapy in patients with RAVs due to previous direct-acting antiviral therapy failure.

P-0179

Regional Differences of HCV and Clinical Characteristics of Chronic hepatitis C in Jeollanam-do

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Background and aims: Data about HCV genotypes distribution of Jeollanam-do are scarce. Therefore, the present study aimed at determining HCV genotypes distribution among 1388 HCV RNA positive individuals from Jeollanam-do province.

Patients and Method: In total, 1388 patients who were positive HCV RNA were analyzed from January 2004 to December 2012.

Results: Out of 1388 HCV RNA positive patients, 794 (57.2 %) were males and were 594 (42.8 %) females. The distribution of genotypes was as follows: 3.6 % of genotype 1a; 43.4 % of genotype 1b; 1.3 % of genotype 1c; 0.9 % of genotype single 1; 4.9 % genotype of 2a; 33.5 % genotype 2a/2c; 8.8 % of genotype single 2; 0.5 % of genotype 3; 0.5 % of genotype 4; 0.9 % of genotype 6; and 1.6 % patients were others. Most notable finding is regional difference of genotype in inland area and coastal area. The genotype 2 was the most frequent genotype in coastal area (55 %; 120/218), compared with genotype 1 in inland area (53.8 %; 224/416). 307 (22.1 %) patients received antiviral treatment. The sustained virologic response (SVR) of enrolled patients was 62.5 %. The SVR rate of genotype 1 was 48.9 %; 75 % of genotype 2; and 57.1 % of others.

Conclusion: The most common HCV genotype in Jeollanam-do is type 1b. The genotype 2 was the most frequent genotype in coastal area compared with genotype 1 in inland area. Regional difference in genotypes was observed in Jeollanam-do province. This study may facilitate treatment options and preventive strategies in Jeollanam-do province.

P-0180

Resistance-associated variants in hepatitis C patients who failed direct acting antiviral treatment

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Backgrounds/Aims: The emergence of resistance-associated variants (RAVs) remains as an unresolved important issue for the treatment with direct acting antiviral agents (DAAs). In this study, we investigated RAVs after the treatment failure with DAA containing regimen for genotype 1 hepatitis C patients.

Patients and Methods: 143 patients with chronic hepatitis C or compensated cirrhosis were treated with PEG-IFN, ribavirin (RBV) and NS3/4A protease inhibitor [triple regimen]. 349 patients were treated with daclatasvir and asunaprevir [IFN-free regimen]. RAVs were detected by cycleave PCR, invader PCR or direct sequencing and the measurements of Y93H strain in NS5A region were performed mainly by cycleave PCR.

Results: Sustained viral response (SVR) rate was 77.6 % in the triple regimen and 88.8 % in the IFN-free regimen. SVR rate was 89.6 %/75.0 %/25.0 % when L31 mutants were negative/weakly positive/positive and 91.5 %/89.4 %/47.1 % for Y93H in the same manner as L31. In 30 non-SVR cases by IFN-free regimen, patients with D168 mutants in the NS3 region increased from 8 to 23 during the treatment. In NS5A region, RAV-positive patients were increased from 4 to 21 for L31 and from 11 to 23 for Y93. Of 7 cases who did not achieve SVR by DCV including regimen (PEG-IFN/RBV/DCV or ASV/DCV), all cases showed RAV-positive in L31 and 6 cases showed positive in Y93 after 3 years of treatment.

Conclusions: Frequency of RAV in non-SVR cases increased after the treatment failure with DAA. RAVs in NS5A region remained for a long period after the treatment with NS5A inhibitor.

P-0181

Significance of Hepatic Insulin Clearance in Patients with CHC and NAFLD

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Background: Hyperinsulinemia plays an important role in the pathophysiological processes of chronic hepatitis C (CHC) and non-alcoholic fatty liver disease (NAFLD). However, there are few reports on hepatic insulin clearance in patients with these diseases.

Methods: A total of 74 CHC patients and 37 NAFLD patients were enrolled in this study. We evaluated their hepatic insulin clearance, insulin sensitivity and β -cell function with an oral glucose tolerance test.

Results: Hepatic insulin clearance in the patients with CHC was significantly correlated with platelets ($r = 0.271$, $p = 0.020$) and liver fibrosis ($r = -0.234$, $p = 0.045$) and was significantly affected by both steatosis (mild, 0.157 ± 0.078 ; severe, 0.114 ± 0.053 ; $p = 0.024$) and fibrosis (mild, 0.167 ± 0.0857 ; severe, 0.125 ± 0.052 ; $p = 0.010$). There were no significant differences in (homeostasis model assessment) HOMA- β among steatosis and fibrosis stages. In the NAFLD patients, those with severe fibrosis had

significantly reduced hepatic insulin clearance (mild, 0.135 ± 0.045 ; severe, 0.098 ± 0.031 ; $p = 0.013$) and significantly increased HOMA- β (mild, 115.6 ± 67.1 ; severe, 172.8 ± 65.7 ; $p = 0.018$) compared with the patients with mild fibrosis.

Conclusion: Liver fibrosis development is associated with hepatic insulin clearance in both the CHC and NAFLD patients.

P-0182

The association between the efficacy of dual oral therapy for HCV and resistance-associated variants

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Background & aims: Direct acting antivirals (DAAs) for hepatitis C virus (HCV) can result in high sustained virologic response (VR) rates. Pre-existing resistance-associated variants (RAVs) of NS3 and NS5A lesions may affect therapeutic efficacy. We therefore examined the therapeutic effect of DAAs in the context of various RAVs.

Methods: We investigated the viral genome sequence by direct sequencing method in 699 patients infected with HCV genotype 1. A total of 322 patients underwent daclatasvir plus asunaprevir (D/A) therapy. The effect of baseline RAVs on response to D/A therapy was analysed.

Results: Of the 699 patients, 351 had been previously treated with interferon-based therapy, and 39 with PEG-IFN plus ribavirin plus protease inhibitor (PR + PI). The incidences of RAVs in NS3 lesions were T54, 2.7 %; Q80, 13.2 %; and D168, 4.5 %; in NS5A they were L31, 4.2 % and Y93, 12.1 %. RAVs T54 and D168 were significantly more common in patients previously treated with PR + PI. RAV Y93 was significantly associated with the IL28B TT genotype ($p < 0.001$). Of patients who underwent D/A therapy, 92.1 % had no baseline RAVs. Patient VR rates were higher in the absence of RAVs than in their presence (86 % (178/207) vs. 70 % (14/20) at week 4, 85 % (68/80) vs. 64 % (9/14) at the end of treatment (EOT), respectively). HCV RNA, RAVs, and the FIB-4 index were all significantly associated with VR at week 4 ($p = 0.027$, 0.036, and 0.026, respectively).

Conclusion: RAV Y93 was associated with the IL28B genotype. Both RAVs and liver fibrosis may be associated with VR.

P-0183

The Value of Cure for Chronic Hepatitis C (CH-C) to the Society

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Background: All oral regimens for CHC have >95 % cure rates. HCV cure leads to improvement quality of life and long-term outcomes including cirrhosis, liver cancer and liver mortality and their associated costs to the society. This study aimed to assess the value of cure with different GT 1 treatment regimens.

Methods: We took the US societal perspective. Treatment eligible GT1 TN patients entered a Markov model which projected quality-adjusted life-years (QALYs) gained over their lifetime. We compared no treatment to approved treatments [2nd generation (sofosbuvir + PR and simeprevir + PR), and all-oral regimens i.e. ledipasvir/sofosbuvir and ombitasvir + paritaprevir/ritonavir + dasabuvir]. Data inputs were based on literature, public sources and consensus by hepatologists; SVR rates were from Phase III trials.

Results: Treating all eligible TN CHC patients in the US with 2nd generation triple, or all-oral regimens were projected to incur drug costs of \$109billion and \$128billion. Using a \$50,000 threshold for the value of a QALY, these regimens were associated with \$129billion and \$198billion savings from HCV cure. Subtracting the cost associated with these regimens from the economic gains of HCV cure yields the value of cure: -\$20billion (2nd generation) and -\$69billion (all-oral). These savings were even greater if the total lifetime costs of CHC complications were added, resulting in value of cure: -\$149billion (2nd generation) and -\$257billion (all-oral).

P-0184

Treatment rates of chronic hepatitis C (CHC): a meta-analysis of 12 studies and 307,031 patients

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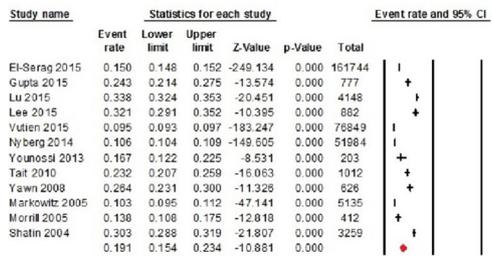
Background: Treatment rates for CHC have not been well characterized. Our goal was to conduct a meta-analysis of HCV treatment rates.

Methods: In 8/2015, we searched MEDLINE, SCOPUS, and the last two meetings of AASLD, APASL, DDW, EASL, and ACG for studies with HCV treatment rates. We excluded studies with >10 % of HIV or HBV co-infection or from a dedicated HCV treatment center. Treatment eligibility was per regional practice guidelines. We used random-effects models to estimate effect sizes.

Results: Twelve studies were included: nine from the US, two from Asia, and one from Europe (Table). Overall, 67 % (CI 47.7–81.5 %) were treatment eligible and 34 % (CI 15.5–60 %) of these were treated. The treatment rate for all CHC patients was 19.1 % (CI 15.4–23.4 %) (Figure) and without gender differences but there was a significantly higher rate in studies of university (28 %) compared with community (13 %) settings ($p < 0.001$). Population-based studies also had a lower treatment rate (18 %) than studies from single centers (22.7 %, $p = 0.335$). The treatment rate was 28.1 % (CI 17.4–42 %) in Asia and 17 % (CI 13.3–21.6 %) in the US ($p = 0.065$), but both Asian studies were from university referral practices.

Conclusions: The overall CHC treatment rate was 19 % and only 34 % of treatment eligible patients received treatment. The literature is very sparse for this important topic, especially in Asia and other non-US regions. Further educational, clinical, and research efforts are needed to optimize CHC treatment rates.

First Author, Year	Country	Study	Population-based	Setting	Treatment rate
El-Serag et al, 2015	US	Abstract	Yes	Mixed	24,248/161,744 (15%)
Gupta et al, 2015	India	Abstract	No	University	189/777 (24.3%)
Lu et al, 2015	US	Abstract	Yes	Mixed	1,403/4,148 (33.8%)
Lee et al, 2015	Korea	Article	No	University	283/882 (32.1%)
Vutien et al, 2015	US	Abstract	Yes	Mixed	7,274/76,849 (9.5%)
Nyberg et al, 2014	US	Abstract	Yes	Community	5,533/51,984 (10.6%)
Younossi et al, 2013	US	Article	Yes	Community	34/203 (16.7%)
Tait et al, 2010	UK	Article	Yes	Mixed	235/1,012 (23.2%)
Yawn et al, 2008	US	Article	Yes	Mixed	165/626 (26.4%)
Markowitz et al, 2005	US	Article	Yes	Mixed	529/5,135 (10.3%)
Morrill et al, 2005	US	Article	No	Community	57/412 (13.8%)
Shatin et al, 2004	US	Article	Yes	Mixed	989/3,259 (30.3%)



P-0185

The effect of DAA antiviral therapy on hepatitis C virus-related thrombocytopenia: a case report

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Background: Immunologically mediated thrombocytopenia has been reported to have association with bacterial and viral infections. Among the viral infections, Hepatitis C virus (HCV) infection has been reported to be associated with the occurrence of ITP. We report the case of a 24-year-old Chinese male student who developed a severe, reversible, immune thrombocytopenia associated with hepatitis C virus infection. We described also our treatment schedule involving DAA therapy of sofosbuvir and daclatasvir on the basis of platelet count and hepatitis C virus-ribonucleic acid (HCV-RNA) levels.

Methods: We followed up the patient during the antiviral therapy of 12 weeks, and at the 12th and 24th week after the end of the treatment. We paid attention to the changes of the serum HCV RNA and platelet count of this patient. Results: In our case, after 2 weeks' DAA treatment, serum HCV-RNA became negative with the lowest limit of 15 IU/ml and stayed undetectable after that. And in the process of DAA antiviral therapy, platelet count gradually increased to normal level with the parallel decrease of HCV RNA.

Conclusions: In our case report, we observed a close relationship between HCV viral replication and thrombocytopenia, and found that DAA antiviral therapy could induce significant improvements in platelet count while clearing the virus. Compared to PEG-IFN/RBV therapy, DAA antiviral therapy may be more suitable for reverting HCV-related thrombocytopenia.

P-0186

Anti-CCP hs in Egyptian rheumatoid arthritis patients associated with chronic HCV infection

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Introduction: Anti-CCP hs (high sensitive) is a highly sensitive and specific laboratory test for the diagnosis of RA and have a role in promoting collagen synthesis in chronic hepatitis C patients. So, the objectives of this study was to evaluate anti-CCP hs in RA patients associated with chronic hepatitis C and its correlation to disease activity and liver affection.

Method: This study was carried out on 90 chronic HCV infection patients, 90 HCV negative RA patients and 90 HCV positive RA patients, in addition to 90 healthy volunteers. Hepatic, rheumatological examination, Quantitative HCV RNA test and abdominal ultrasonography were assessed in all HCV patients. DAS-28 was assessed in RA patients. ESR, CRP, ALT, AST, C4, ANA, Cryoglobulins, RF, anti-CCP3, anti-CCP hs (high sensitive) test were assessed for all patients.

Results: The higher prevalence of anti-CCP hs was found in RA (HCV + ve) compared to RA (HCV-ve) and HCV patients, its sensitivity in RA patients was 75.56 % and specificity was 85.56 %. In HCV patients anti-CCP hs was positively correlated with cryoglobulinemia and scoring for liver fibrosis ($P < 0.001$). In RA patients, anti-CCP hs was positively correlated with RF, anti-CCP3, DAS-28, ESR and CRP ($P < 0.001$).

Conclusions: Serum anti CCP hs is sensitive but not specific marker for RA patients and cannot used as diagnostic marker to differentiate between RA and chronic hepatitis C associated arthropathy, in addition it cannot be used as a maker of activity in RA especially when associated with hepatitis C virus.

P-0187

Inhibition of pannexin 1 channels alleviates acetaminophen-induced hepatotoxicity

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Background: Pannexins constitute a relatively new family of transmembrane proteins that form channels linking the cytoplasmic

compartment with the extracellular environment. A number of reports have documented the presence of pannexin 1 channels in liver, where it underlies inflammatory responses such as occurring upon ischemia-reperfusion injury and experimentally induced non-alcoholic steatohepatitis. In the present study, it was investigated whether pannexin 1 channels equally plays a role in acute drug-induced liver toxicity. For this purpose, a well-established inhibitor of pannexin 1 channels, 10Panx1, was tested for its potential to reduce acetaminophen-induced liver injury.

Methods: Mice were overdosed with acetaminophen followed by treatment with 10Panx1. Sampling was performed 24 h after acetaminophen administration. Evaluation of the effect of pannexin 1 channel inhibition was based on a number of clinically relevant readouts, including serum levels of alanine and aspartate aminotransferase as well as histopathological examination of liver tissue with quantification of necrotic areas. Inflammation was assessed based on hepatic and serum quantities of pro-inflammatory cytokines, while oxidative stress was monitored by measuring liver amounts of oxidized and reduced glutathione. Furthermore, liver regeneration was studied through expression analysis of proliferating cell nuclear antigen.

Results: Most parameters measured point to alleviation of liver cell death and inflammation, changes in oxidized status and concomitant promotion of regenerative activity upon suppression of pannexin 1 channels. **Conclusions:** Pannexin 1 channels are important actors in liver injury triggered by acetaminophen. Inhibition of pannexin 1 channels could represent a novel approach for the treatment of drug-induced hepatotoxicity.

P-0188

Predictors of Acute Liver Failure with Hepatic Coma and its Relationships to Outcome

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Introduction: Difference of etiology of acute liver failure (ALF) between Asian country and US is evident but conflicting. Medical treatment to ALF without liver transplantation (LT) is still mainstream in Japan because of scarce of deceased donor. Additionally, data on predictors of ALF and its timing to perform LT is unremarkable.

Aims: We studied predictors of mortality in ALF patients. Moreover, we examined the predictors related to ALF with hepatic coma.

Methods: A retrospective, analytical study of 167 consecutive, adult patients suspected ALF between Jan 1995 to Sep 2015 in Okinawa Chubu hospital data base. We reviewed all records and excluded 136 cases did not meet new criteria of ALF in Japan. Finally, 30 patients were analyzed in dataset. Data were divided into groups ALF with and without hepatic coma.

Results: Average patient age was 54.1 years old including 17 male patients. A total of 20 patients (67 %) developed ALF with hepatic coma. In this group, 14 patients (70 %) developed ALF with hepatic coma secondary to Hepatitis B related hepatitis including 3 cases of de novo hepatitis B. 25 patients (83 %) were treated with plasma exchange, 21 patients (70 %) with hemodiafiltration. Finally, logistic regression analysis showed two significant predictors of mortality between ALF. Two predictors of mortality were age (OR: 1.069; 95 % CI 0.997–1.146, $p = 0.059$) and liver atrophy (OR: 75.3; 95 % CI 4.89–1171, $p = 0.02$).

Conclusions: Incidence of mortality in ALF with hepatic coma was 80 % without liver transplantation. Elderly patients diagnosed ALF with hepatic coma developing liver atrophy need LT for survival.

P-0189

Acute liver failure caused by valproic acid

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Introduction: Acute liver failure is a rare but life-threatening critical illness, characterized by jaundice, hepatic encephalopathy and bleeding tendency due to impairment of liver function. Drugs are important cause of liver injury. Such injury may be dose dependent and predictable, but it may also be idiosyncratic, unpredictable and probably dose dependent.

Case Report: A 19 year old man with history of seizure admitted to the emergency department with altered mental status. 1 day before he was acutely disoriented and increasingly somnolent. On physical examination he has icterus and mild right upper quadrant tenderness. Laboratory studies are notable for serum ALT of 988 U/L. Total bilirubin 20.09 mg/dl, INR of 5.0. On Head CT scan revealed intracerebral haemorrhagic and sub arachnoid haemorrhagic at right frontal lobe 0.97 cc in size and cerebral oedema. He take valproic acid for his seizure since 2 weeks before He was successfully treated medically with, ceftriaxone 1 gr iv bid, mannitol infusion and L-ornithine L aspartate.

Conclusion: Identifying drug related liver injury is very important because the severity of hepatotoxicity can be decreased if the drug is discontinued. The most important aspect of treatment in patients with acute liver failure is to provide good intensive care.

Keywords: Valproic acid, Acute liver failure.

P-0190

Indication of liver transplantation for acute liver failure with pancreatitis: report of two cases

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Background: Acute liver failure (ALF) is often fatal without liver transplantation. Acute pancreatitis (AP) is seen in patients with ALF not infrequently, but liver transplantation in such condition may be contraindicated due to high mortality. Here we report two cases with AP during evaluation for liver transplantation for ALF.

Case 1: 66-year-old woman, who was diagnosed with acute onset autoimmune hepatitis and under corticosteroid therapy, subsequently developed hepatic encephalopathy. She was diagnosed with ALF and artificial liver support was started. 3 days after the onset of ALF, elevation of amylase at 506 mg/dl and lipase at 696 U/L was observed, and imaging study revealed enlarged pancreas and fluid correction consistent with AP. After improvement of AP under intensive care, immediate living donor liver transplantation (LDLT) was indicated; unfortunately, LDLT was abandoned because of donor issues.

Case 2: A 40-year-old woman with severe hepatic failure from unknown cause was transferred to our hospital. Steroid pulse therapy was started suspecting drug-induced hepatitis. On day 3, hepatic encephalopathy developed, and artificial liver support was started as a treatment for ALF. On day 5, elevation of amylase at 1030 mg/dl and

lipase at 2184 U/L was noted, but imaging study was inconclusive with AP. On day 15, she underwent LDLT from her mother. Serum amylase and lipase levels were normalized at the time of LDLT, and there was no evidence of AP during post-transplantation course.

Conclusion: Patients with ALF complicated by AP may be amenable to liver transplantation under intensive medical care.

P-0191

Factors influencing infectious complications in severe and fulminant hepatitis

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Background: Infection is the most important complication in severe hepatitis (SH) and fulminant hepatitis (FH), influencing indication of liver transplantation. There have been little studies done concerning factors influencing infectious complications. Therefore, we investigated factors influencing infectious complications in SH and FH.

Methods: Eighty patients (42 female, median age 52 years) comprising 42 SH and 38 FH were analyzed. Results: Corticosteroids (CSs) were administered to 63 patients. Thirty-six infectious complications were observed in 25 patients: 21 bacterial, 7 fungal, and 8 cytomegaloviral infections. Median duration from the diagnosis of ALF to onset of infection was 14 days, and that from the introduction of CS to onset of infection 20 days. Accumulative incidence of infection was 3.8 % in 7 days, 5.1 % in 10 days, 14.2 % in 14 days, and 16.9 % in 21 days from the diagnosis of ALF. Accumulative incidence of infection was 1.6 % in 7 days, 6.5 % in 10 days, 18.3 % in 14 days, and 25.7 % in 21 days from the introduction of CSs. Patients with infection were younger ($p = 0.01$) and showed lower AST and ALT levels ($p = 0.02$ and $p = 0.04$), higher T-BIL level and MELD score ($p = 0.001$ and $p = 0.03$). There were no significant differences in clinicopathological factors between patients with and without CS. Accumulative incidence of infection was higher in FH than SH ($p = 0.003$).

Conclusions: Advanced liver failure is risk factor of infectious complications in ALF. CS use dose not influence the occurrence of infection.

P-0192

Acute liver failure caused by mushroom poisoning a case report

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Mushrooms belong to the world of plants, it's various all over the world a, there is about 5000 species of mushrooms, but accounted for only about 15 % is edible, so wild mushroom poisoning into the frequent occurrence of public health events, it is reported that the toxicity of Amanitafun is stronger and it is also common in poisonous mushroom, the poisoning manifestations can be for liver and kidney damage. A 16 year old boy eats poisonous mushrooms occurred liver failure, with onset of acute gastroenteritis, in the middle there is a and pseudo recovery period, followed by abnormal liver function, coagulation disorders, the hepatic coma, after 2 weeks of active treatment

into the recovery period, liver function improved, normal blood coagulation. Summer is the rainy season, wild mushrooms grow rapidly, it's easy to misidentification and eat, therefore wild mushroom poisoning incidents occurred frequently. Recently we made a diagnosis and gave treatment for a number of mushroom poisoning patients in succession in our hospital, some of them is life-threatening, now in 1 case because of edible mushroom after occurrence of liver failure through active rescue successfully treated patients reported, as follows.

P-0193

Transition of etiology in acute liver failure in Hokkaido, Japan-emerging new cause

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Aims: Etiology of acute hepatitis can be changed by sustained hygiene and infection prophylaxis. Acute liver failure (ALF) remained critical burden because of difficulty in treatment. The aim in this study was to clarify transition in ALF etiologies in Japan.

Methods: ALF patients diagnosed from 1998 through June 2015 were reviewed, and causes were assessed every 9 years, in 1998–2006 and 2007–2015. ALF was defined as acute liver injury with PT INR in 1.5 or more.

Results: A total of 127 cases (63 females, 49 median year-old) of ALF during 18 years were enrolled. Causes of ALF were infection of hepatitis viruses in 56 patients (44 %), autoimmune hepatitis with acute presentation (a-AIH) in 16 (13 %), circulatory disorders in 14 (11 %), drugs in 8 (6 %), others in 7 (5 %), and unknown in 26 (20 %), respectively. Among 56 cases of hepatotropic virus infection, 21 were infected with HBV, 19 in HEV, 10 in HAV, 1 in HCV, and 5 in de novo HBV, respectively. In 127 ALF patients, 63 were diagnosed in 98–06, and 64 in 07–15. Hepatotropic virus was causative in 40 (63 %) during 98–06 but in 16 (25 %) on 07–15, and HBV was in 17 (27 %) and 4 (6 %) respectively. On the other hand, A-AIH increased from 4(6 %) to 12 (19 %), and unknown etiology from 7(11 %) to 19(30 %) respectively ($p < 0.001$). Conclusions: The etiological transition in ALF, declining viral hepatitis and emerging a-AIH during the last 18 years, urges hepatologists to make appropriate diagnosis for origin of diseases.

P-0194

Comparison of LdT vs ETV in patients with hepatitis B related ACLF in Bangladesh

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Aim: To compare telbivudine and entecavir in treatment naive hepatitis B virus (HBV)-related acute-on-chronic liver failure (ACLF-B).

Methods: Nineteen patients aged 25–60 years recruited. Informed, written consent obtained. 7 females and 12 male. Acute insult was HBV flare in all. All had detectable HBV-DNA (3.2×10^4 – 1.1×10^7 copies/ml). 13/19 (68.4 %) HBeAg-negative and 6/19 (31.6 %) HBeAg-positive. 12/19 (63.2 %) had ascites only, 5/19 (26.3 %) ascites plus hepatic encephalopathy (HE) and 2/19 (10.5 %) HE only. Serum bilirubin was 6.5–31 mg/dl, ALT 530–4235 U/L, albumin 1.0–2.8 gm/L and INR >1.9. creatinine >1.5 mg/dL in 10 patients. Treatment at presentation were with tablet telbivudine (600 mg) (10/19) or tablet entecavir (0.5 mg) (9/19) orally daily. S. bilirubin, S. albumin, S. creatinine, INR and blood counts monitored weekly for 2 weeks, at 1 month and monthly for 2 months. HBV-DNA monitored at 2 weeks and at end of 3 months.

Results: At 3 months, 8 expired. All had S. creatinine > 1.5 mg/dL at baseline. Of them 2 presented with ascites only, 4 ascites plus HE and 2 HE only. 7/11 surviving were on telbivudine and 4/11 were on entecavir. Three had complete LFT normalization; all receiving telbivudine. LFT improvement was seen in 2 in each arm and remained steady in 4, again 2 each in each arm. HBV-DNA were undetectable in 6 after 2 weeks with 5 getting telbivudine. Nine had undetectable HBV-DNA after 3 months with 6 on telbivudine. None experienced HBeAg-seroconversion.

Conclusion: Study has shown that safety and efficacy of telbivudine and entecavir in ALCF-B with better survival with telbivudine. Study with large patient pool is warranted.

P-0195

Comparison between TDF vs ETV in patients with hepatitis B related ALCF in Bangladesh

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Aim: The aim is to compare tenofovir and entecavir in treatment naive hepatitis B related acute-on-chronic liver failure (ACLF-B) patients.

Methods: Thirty four patients aged 14–65 years recruited and informed written consent obtained from all. 4/34 (11.8 %) were females and 30/34 (88.2 %) males. Acute insult was HBV flare in all. None had HCC. HBV-DNA was 1.8×10^4 – 8×10^8 IU/L at baseline. All had ascites and 11/34 (32.4 %) had hepatic encephalopathy (HE) in addition. None had only HE. At baseline, S. bilirubin was 5.3–33.5 mg/dl, ALT was 50–3028 U/L, S. albumin was 1.8–3 gm/L and INR >1.9. Treatment at presentation was with tablet tenofovir (300 mg) (19/34) or tablet entecavir (0.5 mg) (15/34) orally daily, pending HBV-DNA, planning discontinuation of treatment if HBV-DNA was undetectable. Biochemistry and haematology were monitored weekly for 2 weeks, at 1 month and monthly for 2 months. HBV-DNA was monitored at end of 3 months of treatment.

Results: At end of 3 months, 12/34 (35.3 %) patients expired. Cause of death was hepato-renal syndrome in 11 and sepsis in 1. Among the survivors, 16/22 (72.7 %) had received tenofovir and only 6/22 (27.3 %) were on entecavir. Improvement of MELD score was seen in all survivors on entecavir and in all but 2 on tenofovir at end of follow up. However all who survived had undetectable HBV-DNA after 3 months in both arms.

Conclusion: Present study reconfirms safety and efficacy of both tenofovir and entecavir in ALCF-B with much better survival with tenofovir. However for specific conclusion, further study with larger patient pool is recommended.

P-0196

Serum apoA-V in survival prediction of hepatitis B virus-related acute-on-chronic liver failure

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Aim: Hepatitis B-related acute-on-chronic liver failure (HBV-ACLF) is a life-threatening condition and lipid metabolism disorder is common in the development of disease. The aim of this study was to define the characteristics of serum apolipoprotein A-V (apoA-V) concentration in HBV-ACLF.

Methods: A total of 330 HBV-ACLF patients were recruited in this study, and the relationships of serum apoA-V concentration with clinical variables were analyzed. The independent factors associated with the prognosis of HBV-ACLF were assessed by using the binary logistic regression model, and receiver operating characteristic (ROC) curve analysis was performed to assess the accuracy of serum apoA-V in predicting the survival of HBV-ACLF.

Results: Of the 330 patients, high to 209 patients (63.33 %) died in hospital or after being discharged from hospital. As compared to survivors, the non-survivors had significantly lower concentrations of serum apoA-V; serum apoA-V concentrations were positively correlated with PTA, and negatively correlated with interleukin-10, tumor necrosis factor α , and iMELD scores. Though serum apoA-V, iMELD score and PTA were all independent factors to predict survival, serum apoA-V had the highest performance for the prediction of the survival of HBV-ACLF and the cut-off value of >480.00 ng/mL had a positive predictive value of 84.68 % and a negative predictive value of 92.23 %.

Conclusion: Serum concentration of apoA-V decreases significantly in non-survivors of HBV-ACLF, and serum apoA-V may be regarded as an early predictive marker for the prognosis of HBV-ACLF.

P-0197

Comparison between TDF vs TDF plus GCSF in patients with ALCF-B in Bangladesh

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Aim: The aim is to compare tenofovir and tenofovir-GCSF combination in treatment-naïve HBV-related acute-on-chronic liver failure (ACLF-B).

Methods: Nineteen patients aged 18–62 years recruited and informed written consent obtained. 2/19 (10.5 %) were females and 17/19 (89.5 %) males. Acute insult was HBV flare in all. None had HCC. HBV-DNA was 1.8×10^2 – 5.58×10^6 IU/L at baseline. All had ascites and 8/19 (42.1 %) had hepatic encephalopathy (HE) in addition. None had only HE. At baseline, S. bilirubin was 6–43.4 mg/dl, ALT 36–1102 U/L, S. albumin 1.1–3.8 gm/L and INR >1.9. Treatment at presentation was with tablet tenofovir (300 mg) (9/19) or tenofovir (300 mg) plus GCSF (30 IU) sub-cutaneously daily for 6 days (10/19), pending HBV-DNA, planning treatment discontinuation if HBV-DNA undetectable. Biochemistry and haematology were monitored weekly for 2 weeks, at 1 month and monthly for 2 months. HBV-DNA was monitored at end of 3 months of treatment.

Results: At end of 3 months, 3/19 (15.8 %) patients expired. Cause of death was hepato-renal syndrome in 2 and variceal bleeding in 1. Among the survivors, 9/10 (90 %) received tenofovir-GCSF and 7/9 (77.8 %) received only tenofovir. Improvement of MELD score was in 9/10 (90 %) survivors on tenofovir-GCSF and in 7/9 (77.8 %) on tenofovir only at end of follow up. However all who survived had undetectable HBV-DNA after 3 months in both arms.

Conclusion: Present study reconfirms safety and efficacy of both tenofovir and tenofovir-GCSF in ACLF-B with much better survival with tenofovir-GCSF. However for specific conclusion, further study with larger patient pool is recommended.

P-0198

Down-regulated miR-181c was related with HBV associated acute-on-chronic liver failure by TNFalpha

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Background: Hepatocyte apoptosis induced by tumor necrosis factor (TNF)- α /TNFR1 is an important pathway for the incidence of fulminant viral hepatitis. Accumulating evidence suggests that a limited number of microRNAs (miRNAs) are involved in severe exacerbation of hepatitis B. The relationship between circulating miRNAs and HBV associated acute-on-chronic liver failure (HBV-ACLF) need to be further investigated.

Methods: miRNA expression profile by miRNA microarray analysis was performed on pooled Peripheral Blood Mononuclear Cell (PBMC) obtained from identified groups of patients with chronic hepatitis B (CHB) or HBV-ACLF, respectively. Selected unnormal expressed miRNAs were verified in more clinical samples by quantitative real-time PCR (qRT-PCR). Targets were then subjected to a prediction by bioinformatics target prediction software. A luciferase reporter assay was conducted to confirm whether TNF- α is a direct target of Hsa-miR-181c.

Results: Our results showed 7 kinds of miRNAs were down-regulated and 9 kinds of miRNAs were up-regulated in the PBMC of HBV-ACLF patients by microarray. Expression of Hsa-miRNA-181c was significantly down-regulated in these patients by qRT-PCR. TNF- α was experimentally verified as a target of Hsa-miR-181c.

Conclusion: Our data suggest a potential role for Hsa-miRNA-181c in the regulation of TNF- α expression in patients with HBV-ACLF.

P-0199

Role of Th17 and Treg cells in the progression of grave hepatitis B infection

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Hepatitis B infection poses a severe threat to public health. It remains largely unknown why some patients with grave hepatitis B present with rapid deterioration of liver function, and eventually dies of liver failure while others with similar initial presentations are recovered from the diseases. The current study aims to investigate the role of Th17 and Treg cells in the progression of grave hepatitis B infection. Levels of Th17 and Treg cells in the peripheral blood of 19 patients with signs of clinical deterioration and 21 patients with signs of clinical improvements whose baseline biochemical parameters are comparable and who received similar treatments were determined upon admission, 1 and 2 weeks post-treatment, and the ratio of Th17/Treg cells was calculated. IL-17, TNF-alpha, IL-10 and TGF-beta levels in above samples were also determined with ELISA. We found levels of Th17 cells, IL-17 and TNF-alpha in the peripheral blood and Th17/Treg ratio were positively correlated with the progression of the disease while levels of Treg cells, IL-10 and TGF-beta were negatively correlated. Our results indicate that Th17/Treg cells and their secreted cytokines play an important role in the progression of grave hepatitis B, which determines the outcomes of the patients.

P-0200

The dysregulation of ER stress response in AoCLF patients caused by acute exacerbation of CHB

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Background & Aims: Although endoplasmic reticulum (ER) stress is critical in various liver diseases, its role in acute-on-chronic liver failure (AoCLF) caused by acute exacerbation of chronic hepatitis B (CHB) is still elusive. This study aimed to analyze ER stress responses in the progression of HBV-related AoCLF.

Methods: Normal liver tissues (n = 10), liver tissues of CHB (n = 12) and HBV-related AoCLF patients (n = 19) are used. Electron microscopy of the ultrastructure of the ER was carried out on liver specimens. The gene and protein expression levels of ER stress-related genes were measured. We further analyzed the correlation between the expression levels of ER stress-related molecules and liver injury.

Results: Electron microscopy identified typical features of the ER microstructure in AoCLF subjects. Among the three pathway of unfolded protein responses, the PKR-like ER kinase and inositol-requiring enzyme 1 signaling pathway were activated in CHB subjects and inactivated in AoCLF subjects, while the activating transcription factor 6 signaling pathway was sustained in activated form during the progression of AoCLF; the expression of glucose-regulated protein (Grp)78 and Grp94 were gradually decreased in AoCLF subjects compared to healthy individuals and CHB subjects, showing a negative correlation with serum ALT, AST and TBIL; the ER stress-related apoptosis molecules were activated in the progression of acute exacerbation of CHB.

Conclusions: The dysregulated ER stress response may play a complicated role in the pathogenesis of AoCLF, and a severe ER stress response may predict the occurrence of AoCLF caused by acute exacerbation of CHB.

P-0201

Adipose-derived stem cell administration for cytotoxic T lymphocyte-mediated liver injury in mice

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Aim: Adipose-derived mesenchymal stem cells (ASCs) have known to possess immune-regulatory properties, and the attempts to apply ASC therapy to various inflammatory diseases are now in progress. Fulminant hepatic failure (FHF) is one of the targets for ASC therapy, and the therapy has been proved to be effective in ameliorating liver damage in murine FHF models, such as concanavalin A- and carbon tetrachloride-induced liver injuries. In the current study, we evaluated the effect of ASC therapy on cytotoxic T lymphocyte (CTL)-mediated liver injury, most prevalent cause of FHF in human, in order to assess actual usefulness of ASC therapy for FHF.

Methods: Hepatitis B surface antigen (HBsAg)-transgenic mice (HBs-Tg) which retains HBsAg in their hepatocytes, and syngeneic HBsAg-specific CTLs (HBs-CTLs) were used in the study; the HBs-Tg has been shown to develop FHF-like liver injury with the intravenous transfer of HBs-CTLs.

Results: When the mice were transferred with 5×10^6 CTLs alone, serum ALT levels on day 2 were 3700 ± 2107 U/L. When they were transferred with 1×10^6 ASCs 30 m before CTL transfer, ALT levels were 5713 ± 3543 U/L, indicating that ASC transfer did not lessen liver damage. Histological analysis also showed that ASC transfer did not relieve liver inflammation. Furthermore, in vitro study indicated that ASCs did not affect cytotoxic activity of CTLs.

Conclusions: The use of ASCs for FHF should be careful, since their immune-regulatory functions may not be exerted as expected depending on the etiology of FHF.

P-0202

Comparison of different models in predicting prognosis of HBV-ACLF

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Objective: To investigate the predictive value of six models for short- and long-term prognosis in patients with HBV-associated acute-on-chronic liver failure (HBV-ACLF).

Methods: Liver and renal function, electrolyte, prothrombin time and HBV markers of a cohort of 232 HBV-ACLF patients were detected, complications were recorded, and the model for end-stage liver disease (MELD), MELD-Na, integrated MELD (iMELD), child Turcotte Pugh (CTP), modified CTP (mCTP) and logistic regression model (LRM) were calculated. The survival rate of 90 days and 5 years were followed up.

Results: All six models could adequately describe the data and had a significant correlation with the prognosis of HBV-ACLF. Among these models, only LRM had AUC (area under the receiver operating characteristic curve) values exceeding 0.8 at both time points (90-day and 5-year), indicating best predictive accuracy. The optimal cut-off value of LRM had the best discrimination for predicting the outcome risk. The overall performance of LRM, assessed with Nagelkerke's R2 and the Brier score, was also the best among the models.

Conclusion: LRM may be the best model to predict short- and long-term prognosis in HBV-ACLF patients, which may have clinical implications for diagnosis and treatment.

P-0203

Thromboelastography accurately interprets coagulation failure in patients with ACLF

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Background: Coagulation system is rebalanced in cirrhosis and liver failure. The status of baseline coagulation disturbances and their alterations in ACLF are largely unknown.

Aim: This study aimed at determining the prevalence severity and dynamic coagulation profile changes by serially measuring coagulation factor assays, thromboelastography (TEG) in non septic patients with ACLF.

Methods: Consecutive non septic ACLF patients were assessed at days 0, 3, and 7 for coagulation changes using prothrombin time (PT), activated prothrombin time (APTT), and international normalized ratio (INR). Sonoclot and TEG parameters were used for global coagulation assessment. Coagulation assays for factor VIII, vonWillebrand factor (vWF), protein C and antithrombin III were performed.

Results: One hundred fourteen patients with ACLF were recruited (mean age 44.3 ± 11.7 years, males 90 %); predominant aetiology being ethanol (63.1 %). A deranged TEG at presentation was a predictor of bleeding (OR 2.1, $p = 0.05$) and mortality (OR 3.1, $p = 0.05$). Platelet count and functions (as predicted by sonoclot) were significantly lower in ACLF patients with sepsis at day 3 and 7. INR at baseline was associated with a deranged R and K component of TEG ($p = 0.05$) and ACT of Sonoclot ($p = 0.02$). Coagulation factor assays for protein C, antithrombin III, vWF were lower in all ACLF patients, when compared with controls. Factor 8 levels were significantly increased in all ACLF subgroups. See Figure 1.

Conclusions: Thromboelastography and Sonoclot are useful adjuncts for prediction of bleeding events. Coagulation abnormalities in ACLF are associated with an increased tendency to bleed, risk of sepsis and mortality.

P-0204

A case of severe sclerosing cholangitis following critical ill with extensive burn injury

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Aim (Background): Sclerosing cholangitis in critically ill patients (SC-CIP) is a relatively new entity with rapid progression and poor

prognosis. We herein report a case of SC-CIP following extensive burn injury.

Methods (Case presentation): A 70-year-old male patient was referred to our department with jaundice and cholestatic liver biochemistries 4 weeks after severe burn injury while still in the intensive care unit (ICU). He had been treated in ICU due to hemodynamic instability requiring mechanical ventilation and catecholamine administration. The patient had no preexisting hepatobiliary disease, and screening for chronic liver disease was negative. At the first examination, ultrasonography and computed tomography demonstrated no evidence of biliary obstruction. Administration of ursodeoxycholic acid and antibiotics could not improve cholestasis in the patient. Two months after his referral from ICU, endoscopic retrograde cholangiopancreatography showed multiple strictures and dilatations of intrahepatic bile ducts. Liver biopsy revealed severe cholestasis and cholangiopathy. On the basis of these findings, the patient was diagnosed with SC-CIP. Unfortunately, his disease has progressed rapidly after the diagnosis of SC-CIP and he has been repeatedly hospitalized in the past year.

Conclusion: It is important to recognize that SC-CIP is a severe complication in patients who are recovered from life-threatening illnesses requiring aggressive intensive care treatment.

P-0205

Acute on chronic liver failure induced by hepatic injury is characterized by massive cell death

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Background: Acute-on-chronic liver failure (ACLF) is a clinical entity which is distinctive from acute decompensated cirrhosis (ADC). Currently, much is unknown about ACLF. This study aims to investigate the role of hepatic cell death in ACLF.

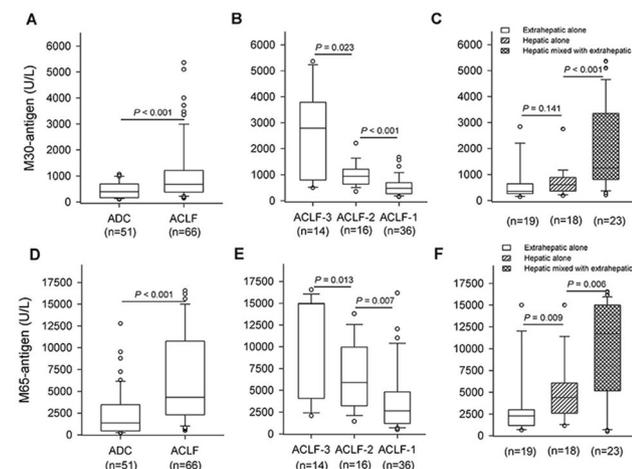
February, 2013 to August, 2014. ACLF was diagnosed as per EASL-CLIF criteria. This criteria was also used to subdivide the ACLF into three grade, ACLF-1, ACLF-2 and ACLF-3. The higher the grade is, the more organ fails. Hepatic cell death was assessed by the measurements of serum M30 (apoptosis) and M65 (total cell death) levels. **Results:** Serum M30- and M65-antigen were significantly higher in ACLF than ADC ($P < 0.001$). Patients with ACLF-3 presented the highest levels of M30- and M65-antigen followed by ACLF-2 and ACLF-1. Significant correlations ($P < 0.001$) were found between M30- or M65-antigen and disease severity scores, CTP, MELD or CLIF-SOFA. ACLF was further categorized into two distinct groups according to the types of acute insults (hepatic or extrahepatic). Extrahepatic-ACLF group demonstrates relative low level of apoptosis ($P > 0.05$) and significant lower total cell death ($P < 0.001$) compared with hepatic-ACLF and were even close to that from ADC ($P > 0.05$). However, cell death biomarkers markedly elevated in the ACLF group mixed with hepatic and extrahepatic insults.

Conclusions: ACLF demonstrates massive hepatic cell death which is absent from ADC. Acute hepatic rather than extrahepatic injury alone leads to such high level of cell death. But extrahepatic insults help exaggerate the hepatic cell death in ACLF patients with acute hepatic injury.

Table 1. Correlation between cell death biomarkers and clinical parameters and scores

	M30-antigen		M65-antigen	
	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>
ALT	0.462	<0.001	0.454	<0.001
AST	0.558	<0.001	0.562	<0.001
TB	0.423	<0.001	0.51	<0.001
INR	0.328	<0.001	0.388	<0.001
Cr	0.121	>0.05	0.145	>0.05
MAP	-0.0763	>0.05	-0.1	>0.05
CTP	0.511	<0.001	0.565	<0.001
MELD	0.415	<0.001	0.489	<0.001
CLIF-SOFA	0.478	<0.001	0.551	<0.001

Abbreviation: TB, total bilirubin; Cr, creatinine; MAP, mean arterial pressure; Child-Turcotte-Pugh; MELD, Model for end-stage liver disease; CLIF-SOFA, chronic liver failure-sequential organ failure



Methods: 66 ACLF patients were identified from a prospective cohort of 117 patients with ADC admitted to Rui-Jin Hospital from

Table 2. Category of acute insults for all the ACLF patients

Events	Frequency
Hepatic insults alone	18 (27.3%)
Reactivation of HBV	12 (18.2%)
Alcoholic hepatitis	3 (4.5%)
Flare up of AIH	1 (1.5%)
Hepatotoxic drugs	2 (3.0%)
Extrahepatic insults alone	18 (27.3%)
Bacterial infection	12 (18.2%)
UGIB	6 (9.1%)
Hepatic mixed with extrahepatic insults	23 (34.8%)
Unknown	6 (9.1%)

Abbreviation: upper gastrointestinal bleeding

P-0206

A case of postoperative hepatic encephalopathy after total cavopulmonary connection conversion

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We experienced a case of postoperative hepatic encephalopathy (HE) developed without a motive after total cavopulmonary connection (TCPC) conversion for original (APC) Fontan circulation.

The patient was 53-years-old man of tricuspid atresia. He was performed APC Fontan operation when he was 19-years-old. The post-operative course was good, but since he was 33-years-old, he developed stroke by thrombosis, caused by right atrium (RA) expansion and atrial fibrillation. Cardiac insufficiency was exacerbated in spite of conservative treatment, he was considered to operation adaptation of TCPC conversion. He had cirrhosis and his laboratory data was; platelet 184,000, TP 7.7, Albumin 4.2, AST 17, ALT 9, γ -GTP 90, and considered tolerable.

TCPC conversion operation using extra cardiac venous graft with fenestration and RA plication. He entered ICU on intubation. He was able to extubate on POD5. On POD6 he shown somnolence tendency, quadriplegia, and could not follow the order. The head CT was performed to show any significant cerebral hemorrhage or stroke. He diagnosed as HE. Lactulose, branched chain amino acid, and levocarnitine were administered. He became awake on POD10 and completely recovered on POD14. He moved to ward on POD16, undertaken cardiac rehabilitation, stopped medication for HE on POD36, and left the hospital on POD77. Seven months has passed from operation, he is followed as an outpatient and taking good course.

Univentricle (Fontan) circulation often induces cirrhosis. By improvement of surgical outcome of Fontan procedure, opportunity of cardiac operation for patients under decades of Fontan circulation will increase. More sensitive preoperative evaluation of liver is needed.

P-0207

Acute-on-chronic liver failure development correlate with baseline portal hypertension

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Background and aim: Acute-on-chronic liver failure (ACLF) is considered as main prognostic event in cirrhosis. Portal hypertension (PHT) and liver disease severity have been accepted as main prognostic factors in the development of acute decompensation which is essential precondition of ACLF. The aim of this study is evaluation about the relationship between baseline PHT, liver disease severity with the future ACLF development.

Methods: 602 Cirrhotic patients (male 499, 82.9 %) have been prospectively followed for the development of ACLF and ARM. Baseline hepatic venous pressure gradient (HVPG) measurement, serologic tests were performed to all patients. The diagnosis of ACLF was based on EASL/AASLD criteria.

Results: 127 (21.1 %) patients developed more than once of ACLF. ACLF related mortality (ARM) was developed in 98 patients (16.3 %). In the univariate analysis, Child-Pugh score (CPS), MELD score, HVPG, CPS class C, MELD score over 15, clinically significant portal hypertension (CSPH), alcohol, total bilirubin (TB), albumin, INR, sodium, hemoglobin were significant for ACLF developments ($P < 0.05$). In the multivariate analysis modeling with avoiding colinearity, CSPH is always significant predictor variables for ACLF development in multiple models. In Kaplan–Meier analysis, CSPH, MELD score over 15, CPS class C were significant for ACLF development ($P < 0.001$). In univariate analysis, CPS, MELD score, HVPG, CSPH, TB and INR were statistically significant for ARM ($P < 0.05$). In multivariate analysis, CPS, MELD score, CSPH and TB were significant for ARM ($P < 0.05$).

Conclusions: Our data suggests that the development of ACLF and ARM were closely related with underlying liver disease severity (CPS, MELD score) and baseline clinically significant portal hypertension.

P-0208

Significant factors for mortality in patients with acute decompensation of chronic liver disease

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Background/Aim: Non-negligible mortality rate has been reported in acute decompensated (AD) patients without ACLF. Nevertheless, data for significant factors for prognosis is limited. We aimed to evaluate the prognostic factors for 90-day mortality in the patients who were admitted for AD of chronic liver disease without ACLF.

Methods: Among the 1470 patients who were admitted for AD of chronic liver disease in 21 university hospitals in Korea in 2013, 902 patients who did not develop ACLF for 1 year were included in this study. ACLF were defined by either APASL or EASL/AASLD guidelines. The training set included 602 patients and significant variables were selected by multiple logistic regression analysis. These results were internally validated in 300 patients of test set.

Results: The 90-day mortality rates in the training and the test set were 6.31 and 6.00 %. Age (OR 1.06, $p = 0.0003$), etiology of HBV combined with alcohol (OR 4.10, $p = 0.0085$), WBC (OR 4.21, $p < 0.0001$), and total bilirubin (OR 2.25, $p < 0.0001$) were the

significant factors in predicting 90-day mortality [Korean AD score = age \times 0.06 + (HBV + Alcohol) \times 0.71 + Ln (WBC/1000) \times 1.44 + Ln (Total bilirubin) \times 0.81]. The AUC for prediction of mortality in test set was 0.763 (95 % CI 0.647–0.879) which were not significantly different from CLIF-C-ACLF score.

Conclusions: This is the first study for the prognosis of Korean AD patients without ACLF for 1 year without regard to etiologies of chronic liver disease. Older age, the etiology of HBV combined with alcohol, leukocytosis, hyperbilirubinemia may be important factors in the discrimination of high-risk group of patients among the hospitalized AD patients.

P-0209

ACLF: clinical profile and factors affecting outcome

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Background and aims: ACLF is defined as an acute deterioration of liver function in a patient with previously fairly well compensated chronic liver disease. The aims of this study were to understand the natural history, etiology of acute insult and underlying chronic liver disease and prognostic outcomes in ACLF.

Material and methods: This prospective study was conducted in the Gastroenterology Department, Institute of Medical Sciences, BHU, Varanasi from December 2013 to November 2014. Data including demographics, clinical presentation and outcome of 86 patients was recorded. The etiology of superimposed acute event and chronic liver disease was investigated on the basis of routine investigations, viral markers, autoimmune markers, Wilson disease panel and serum ferritin. Study variables included coagulopathy, hepatic encephalopathy, sepsis, hyponatremia, renal failure, hepatorenal syndrome and various prognostic scores.

Results: Most of the patients were young adults (55 %) and males were more common than females (M:F = 2.7:1). The common presenting features were coagulopathy and ascites (95 %) and acute insult was hepatotropic viruses (about 50 %). At admission, about 2/3rd patients had CTP-C status. sepsis (20 %), renal failure (13 %) and SBP were common complications. At 3 months, survival of patients with meld $<$ 26 and $>$ 26 was 82 and 31 %, respectively.

Conclusion: Most of the 86 patients with ACLF were young adult males. The acute insult was primarily due to hepatotropic viruses (HAV, HBV and HEV) whereas chronic was due to HBV infection. SOFA was found to be an independent predictor of mortality whereas meld had better accuracy in predicting short term survival.

P-0210

Human mesenchymal stems-engineered hepatic cell sheets therapy for acute liver injury

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Mesenchymal stem cells (MSCs) themselves can transdifferentiate into hepatocytes as well as stimulating liver regeneration by humoral

factors. Therefore, cell-based therapies utilizing MSCs are expected to be an alternative therapy for liver disease. We previously identified hexachlorophene as an inducer of hepatic differentiation of MSCs through downregulation of Wnt/ β -catenin signals. To clarify therapeutic efficacy of hexachlorophene-induced hepatic cells, hepatic cells were transplanted onto liver of liver injury mice model as cell sheets. First, hepatic cell sheets were compared with MSC sheets on therapeutic efficacy, where MSCs were cultured on PIPAAm-grafted dishes treated with or without hexachlorophene. Subsequently, the cell sheets were transplanted onto liver surface of the mice of acute liver injury caused by administration of CCl₄. Compared to MSC sheets, hepatic cell sheets significantly reduced serum transaminases. Transplantation of hepatic cell sheets clearly improved serum transaminases, bilirubin and survival rate depending the numbers of received cell sheets. Importantly, hepatic cell sheets ameliorated serum transaminases in both cases of transplantation of cell sheets after administration of CCl₄ and transplantation of cell sheets before administration of CCl₄. Hepatic cell sheets enhance liver regeneration. Hepatic cell sheets-derived complement C3 was highly expressed and its downstream signals including C5a, NF- κ B, and IL-6/STAT-3 pathway was activated in hepatic cell sheets-grafted tissues. EGFR was also activated. Furthermore, the proteins involving thioredoxin oxidation reduction cycles were enhanced. In conclusion, human mesenchymal stems-engineered hepatic cell sheets therapy is effective for acute liver injury.

P-0211

Comparison of the acute on chronic liver failure severity score: a need for simple and dynamic one

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Background and aim: Acute on chronic liver failure is rapidly progressive liver failure with high short term mortality. A simple and dynamic prognostic model is needed for early and definitive therapy. Aim was to compare the existing disease severity scores for prediction of 90 days survival in ACLF patients by APASL definition .

Method: 1021 ACLF patients' enrolled into the APASL ACLF Research Consortium (AARC) were analyzed. CTP, MELD, SOFA, CLIF SOFA, APACHE II and number of organ failure at baseline and delta change on D4 and D7 were compared with the new onset HE or AKI, change in lactate or bilirubin for prediction of 90 days mortality.

Results: The baseline MELD, CLIF SOFA, APACHE II, SOFA, CTP and number of organ failure as mortality predictor had AUROC of 0.74, 0.72, 0.71, 0.71, 0.68 and 0.68, respectively. Their delta change at D4 or D7 were poor predictor of outcome with AUROC <0.68. The new onset AKI [HR: 2.58 (1.76–3.75)], increase in total bilirubin by 6.1 mg/dl [HR: 2.16 (1.77–2.61)], new onset HE [HR: 2.14 (1.36–3.34)], increase in lactate by 1.48 meq/l [HR: 1.85 (1.45–2.34)] on D4 and D7 had a better hazard for mortality than SOFA [HR: 2.13 (1.41–3.20)], MELD [HR: 1.63 (1.33–1.99)], CLIF-SOFA [HR: 1.42 (1.06–1.90)], CTP [HR: 1.35 (1.07–1.69)] and APACHE II [HR: 1.81 (1.65–2.22)] change.

Conclusion: The existing disease severity score were poor predictor of outcome and lack dynamicity. The change in bilirubin, lactate with development HE and/or AKI were good predictors. A model considering above is strongly recommended.

Table 1: Disease severity score at baseline as predictor of 90 day mortality (AUROC)

	AUROC	Cut off	Sensitivity	Specificity
MELD	0.737	30.5	68.1	68
CLIF SOFA	0.720	12.5	66.7	64.2
APACHE II	0.711	14.5	70.8	64.2
SOFA	0.708	9.5	63.9	64.1
CTP	0.683	11.5	72.2	57.5
No of Organ Failure	0.682	>2	47.5	79.2

Table 2: Dynamic Parameters

Prognostic Parameters	Day 4	P Value	Day 7	P Value
New onset AKI	2.58(1.76-3.74)	<0.001	1.95(1.23-3.09)	0.004
Delta Total Bilirubin	2.16(1.77-2.61)	<0.001	1.68(1.34-2.10)	0.039
New onset HE	2.14(1.36-3.34)	<0.001	1.87(0.86-2.85)	0.039
Delta Lactate	1.85(1.45-2.34)	<0.001	1.55(1.14-2.10)	0.005
SOFA	2.13(1.41-3.20)	<0.001	1.75(1.08-2.84)	0.022
APACHE-II	1.81(1.65-2.22)	0.005	1.42(1.39-1.90)	0.171
MELD Score	1.63(1.33-1.99)	<0.001	1.72(1.36-2.15)	<0.001
CLIF-SOFA	1.43(1.06-1.90)	0.017	1.32(0.90-1.93)	0.145
CTP score	1.35(1.07-1.69)	0.009	1.48(1.16-1.90)	0.002

P-0212

Neutrophil-lymphocyte ratio, an artificial liver support system and prognosis

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Background: Hepatitis B virus-related acute-on-chronic liver failure has high short-term mortality. Artificial liver support systems (ALSS) may improve outcome and avoid liver transplantation, but predicting short-term prognosis in such patients is difficult. This study aimed to determine whether the neutrophil-lymphocyte ratio (NLR), an inflammation marker, predicted mortality in patients treated with ALSS.

Methods: A total of 560 patients with hepatitis B virus-related acute-on-chronic liver failure were enrolled, 338 were treated with ALSS

and the others treated with standard medical therapy (SMT). Clinical variables and the NLR were evaluated for prognostic value.

Results: Thirty-day mortality was 28.4 % in ALSS and 55.4 % in SMT patients. The NLR was lower in survivors than in ALSS or SMT patients who died. Univariate and multivariate analysis found that NLR and Model End-stage Liver Disease scores were independently associated with 30-day mortality. Among patients with NLRs <3, 3–6, and >6, 30-day mortality was 8.3, 22.5, and 72 % in ALSS and 24, 49.5, and 77.4 % in SMT patients. Among patients with NLRs <3 or 3–6, mortality was lower in ALSS than in SMT patients (P < 0.01). Mortality rates of ALSS and SMT patients with NLRs >6 did not differ (P = 0.41). The results suggest that liver function in most patients with NLRs <3 recovered with ALSS treatment, and patients with NLRs >6 needed emergency liver transplantation.

Conclusion: NLR was an independent predictor of mortality in ALSS patients and may assist physicians in determining treatment options.

P-0213

Liver transplantation for patients with acute-on-chronic liver failure in Asia

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Aim: Acute-on-chronic liver failure (ACLF) is characterized by high mortality. Liver transplantation (LT) is effective in patients who do not improve with supportive measures. This study examines the outcome of ACLF patients who underwent LT in Asia.

Methods: Prospectively collected data from 17 Asian countries in the APASL ACLF Research Consortium was analyzed. 43 patients who underwent LT for ACLF were compared with 1657 non-transplanted ACLF patients. The variables analyzed include patient demographics, acute insult, background liver disease, severity scores (MELD and SOFA scores) and post-LT outcome.

Results: Mean age of LT patients was 42.1 years and non-transplanted patients was 43.7 years. 74.4 % of LT patients and 85.1 % of non-LT patients were male. The most common acute liver insult was HBV reactivation (24.4 %) in LT patients, compared with alcohol (49.5 %) in non-LT patients. Three-month survival rate was 76.7 % in LT group, and 52.6 % in non-LT group. Mean MELD scores prior to transplant was (27.7 ± 4.7) and (30.5 ± 8.3) in non-transplant group. In LT patients, baseline renal dysfunction predicted mortality (mean urea: 1.4 vs. 0.84 mg/dL, $p = 0.015$) (mean creatinine: 61 vs. 27 $\mu\text{mol/l}$, $p = 0.042$). High SOFA score was significantly associated with mortality in both LT (12.5 vs. 8, $P = 0.015$) and non-LT (8.3 vs. 10.9, $p < 0.001$) patients. In non-LT patients, baseline urea (68.5 vs. 41.2 $\mu\text{mol/l}$, $p < 0.001$), MELD (33.8 vs. 27.5, $p < 0.001$) and Child-Pugh score (12 vs. 11, $p < 0.001$) were independently associated with mortality.

Conclusion: Baseline renal dysfunction and higher SOFA score predict poorer LT outcome in ACLF patients.

P-0214

Presence of SIRS and sepsis predicts mortality in patients with acute-on-chronic liver failure

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Background: Acute-on-chronic liver failure (ACLF) is characterized by organ failure(s) and high short-term mortality. This study develops and validates a specific prognostic score for ACLF patients.

Methods: Data from 1023 patients included in the prospective ACLF-AARC database were used. Prognosis prediction was done using established models of CLIF-SOFA, MELD, MELD-sodium (MELD-Na), and Child-Pugh (CPs) scores. The level of significance was set at 0.005.

Results: The database has enrolled 1023 patients with mean age 44.2 years (males 88.4 %) with predominant etiology being ethanol. Clinical predictors of mortality at baseline included presence of hepatic encephalopathy >grade 2 [Hazard ratio 4.3 (CI 3.4–5.5)], systemic inflammatory response syndrome [HR 2.5 (CI 2.2–3.2)], septic shock [HR 6.42 (CI 5.9–6.5)]. The presence of spontaneous bacterial peritonitis [HR 1.48 (CI 1.3–1.51)] was an independent predictor of mortality, the other sites of sepsis being pneumonia, urinary tract and skin and soft tissue infection. The new prognostic formula elaborated was as follows: ACLF Prognostic Index (API) = $0.02 \times \text{total bilirubin (mg/dl)} + 0.29 \times \text{INR} + 0.17 \times \text{lactate (mmol/l)} + 1.3$ (if MAP <70 mmHg at baseline) + 0.46 (if serum creatinine >1.5 mg/dl at baseline). The area under the receiver operating characteristic curve of the API in predicting the outcome of patients with ACLF was 0.82, as opposed to 0.71 for the original MELD, 0.72 for MELD sodium and 0.78 for CLIF-C score ($P < 0.05$).

Conclusion: The presence of SIRS and sepsis at ACLF diagnosis predicts mortality. A simple clinically relevant prognostic score can be used to stratify the risk of mortality in ACLF patients.

P-0215

Impact of grade I ACLF on 1-year survival in cirrhotic patients with first acute decompensation

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Background: Short-term mortality rates of patients with grade I ACLF according to EASL-CLIF definition are known to be higher than those of patients without organ failure (No ACLF). We aimed to compare the long-term mortality rates between no ACLF group and grade I ACLF group among the patients who survived more than 6-months, and 1-year.

Methods: Among the 1470 acute decompensated patients (retrospective KACLIF cohort) who were admitted to 21 university hospitals in Korea between Jan. 2013 and Dec. 2013, cirrhotic patients, without prior decompensation, were selected. No ACLF group were 809 patients and grade I ACLF group were 55 patients. Long-term transplant-free survival was monitored until Sep. 2015.

Results: Among the 595 patients who survived more than 6-months, 74 out of 563 patients in no ACLF group, and 8 out of 32 patients in grade I ACLF group died. Transplant-free survival were 31.0 and 24.5 months in no ACLF group and grade I ACLF group, respectively ($p = 0.010$). Among the 406 patients who survived more than 1-year, 49 out of 388 patients in no ACLF group, and 6 out of 18 patients in grade I ACLF group died. Transplant-free survival were 32.1 and 25.8 months in no ACLF group and grade I ACLF group, respectively ($p = 0.005$).

Conclusions: Even with fair outcome in grade I ACLF group of patients, the impact of organ failure persisted more than 1 year. Red flag should be raised on grade I ACLF group even if they had overcome the episode of organ failure.

P-0216

The prognosis of patients with acute on chronic liver failure who were admitted to ICU

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Background: ACLF is a characterized by acute decompensation of cirrhosis, organ failures and high short term mortality.

Material and method: From 2013/1/1 to 2013/12/31, patients who were admitted to our specialized GI intensive care unit due to ACLF

were enrolled for our studies. Various parameters of seven different time points, including ICU 8–14 days were recorded for analysis.

Result: 93 patients fulfill our inclusion and exclusion criteria. The mean in-hospital days were 33.3 ± 28.5 days. The mean follow-up days were 223.9 ± 268.1 days. The 28 day mortality rate was 24.7 %. The in-hospital mortality rate was 52.7 %. The diagnosis day 3–7 ACLF grade could predict 28 day mortality with AUROC area 0.841. However, for the prediction of in-hospital mortality, ICU 8–14 day had highest AUROC area 0.844. Acute kidney injury and hepatic encephalopathy grade could predict in-hospital mortality independently in addition to CLIF C ACLF scores.

Conclusion: The diagnosis day 3–7 ACLF grade could predict 28 day mortality. ICU 8–14 day ACLF grade could predict in-hospital mortality the highest among different time points. Acute kidney injury and hepatic encephalopathy grade could predict in-hospital mortality independently in addition to CLIF C ACLF scores.

P-0217

Autoimmune hepatitis patients positive for anti-mitochondrial M2 antibody

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Aim: Anti-mitochondrial M2 antibody (AMA-M2) is specific to primary biliary cirrhosis (PBC) but can also be found in some autoimmune hepatitis (AIH) patients. Titers of AMA-M2 were analyzed before and after follow-up in patients with PBC or AIH.

Materials and methods: Patients who underwent liver biopsy and were diagnosed with either AIH (ten patients) or PBC (three patients) were enrolled in the study. Their AMA-M2 antibody titers were analyzed upon hospital admission. AMA-M2 reacted with the pyruvate dehydrogenase complex-E2 (PDC-E2), branched-chain 2-oxo acid dehydrogenase complex, and 2-oxoglutaric acid dehydrogenase complex in the assay utilized for this study. The cut-off value of AMA-M2 was 5.

Results: Six AIH patients were AMA-M2(–) and four AIH patients were AMA-M2(+). In the AIH patients who were AMA-M2(+), the titer was 24.8 ± 14.8 . The titer was 324 ± 174 in the PBC patients ($P = 0.0138$). Three AMA-M2(+) AIH patients were followed up after liver biopsy. The AMA-M2 levels had decreased in all three patients, becoming undetectable in two of them.

Conclusion: AMA-M2 was positive in some AIH patients, but the titers were significantly lower than those in PBC patients. Additionally, AMA-M2 titers were decreased in the AIH patients at follow-up.

P-0218

Clinical outcomes of autoimmune hepatitis in North-East Scotland

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Background: Autoimmune hepatitis (AIH) is an idiopathic chronic hepatitis characterized by hyperglobulinaemia, presence of autoantibodies and characteristic lymphocytic inflammation on liver histology. This study aims to outline the clinical course of AIH in a cohort of patients from Aberdeen in North-East Scotland.

Methods: Retrospective analysis of electronic records of 76 patients with a clinical diagnosis of AIH.

Results: The mean age of the patients was 49.6 and 87.2 % were female. Jaundice was the predominant presenting symptom (35.9 %) with 98.7 % noted to have abnormal liver function tests at presentation. 25.6 % of subjects had other associated autoimmune diseases, with 9.21 % having hypothyroidism/thyroiditis. The mean MELD was 10 and 75 % were Child's A at diagnosis. Interface hepatitis was seen in 67.1 %, portal lymphocytic infiltrates (37.3 %), rosettes (3.95 %) and lymphocytic infiltration in 93.6 % of patients. 46.1 and 9.33 % of patients had pathological evidence of fibrosis and cirrhosis respectively. The mean simplified AIH score in this cohort was 6.59. 41 % of patients were treated with steroids alone and 49 % were treated with a combination of steroids and azathioprine. 74.6 % of patients achieved complete treatment response at 6 months with no difference noted between patients with and without cirrhosis ($p = 0.66$). Relapsing pattern was seen in 11.3 % of cases and 5.63 % had refractory disease.

Conclusions: AIH has a female preponderance in this Scottish cohort with an expected benign presentation and progression. A large proportion of patients had excellent response to immunosuppression highlighting the need for early recognition and treatment of this.

P-0219

Incidence and prevalence of autoimmune hepatitis in the Ueda area, Japan

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Background and aims: Although autoimmune hepatitis (AIH) is considered to be rare in Japan, precise data on the incidence and prevalence of this disease are scarce due to the lack of a nationwide registry. We therefore conducted a study of these factors over a secondary medical care area.

Methods: We retrospectively investigated the medical records of AIH patients seen during 2004–2009 and prospectively recruited subjects from 2010 to 2014 at our hospital. We surveyed via written questionnaires to all family doctors and hospitals in our secondary medical care area of Ueda, with a population 187,205 individuals over 14 years of age. We also surveyed several core liver disease hospitals in the areas neighboring Ueda.

Results: Forty-eight patients with AIH were diagnosed between 2004 and 2014. The average annual incidence of AIH in the area was 2.33. Forty-eight patients (37 patients diagnosed between 2004 and 2014, and 11 patients before 2004) were followed to the study end point. The prevalence was 25.6 on December 31, 2014. These values tended to be higher than published worldwide data. AIH with acute presentation was also increased, with a greater number of patients exhibiting histological features of acute hepatitis.

Conclusion: The incidence and prevalence of AIH in Japan may be higher than previously believed due to increased awareness among family doctors, and a rise in the diagnosis of mild or atypical AIH.

P-0220

Clinical presentations of autoimmune hepatitis

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The spectrum of clinical manifestations of autoimmune hepatitis (AIH) is variable. Approximately 25 % of patients with AIH present with acute onset and 25 % of patients have cirrhosis at the time of diagnosis. Some patients could be asymptomatic and diagnosis was established during the investigation of elevations of the transaminases in asymptomatic patients. In this study, we want to investigate initial symptoms and clinical presentations at the time of diagnosis of AIH in our patient population.

Method: We investigated initial symptoms and clinical presentations in patients (pts) with AIH who were followed and database in our Hepatology Clinic, retrospectively.

Results: Eighty-one pts (F/M:72/9, mean age 54 + 146) with AIH were included. Nineteen patients (23 %) were asymptomatic. We detected nonspecific symptoms like fatigue, myalgia, arthralgia in 46 (56 %), jaundice in 36 (44 %), pruritus in 6 (7.4 %), right upper quadrant pain in 10 (12.3 %) and ascites in two pts. Initial presentations were acute hepatitis in 34 (41 %) pts. Cirrhosis was detected in 13 (16 %) pts. Sixteen of the patients (19.8 %) had also other extra-hepatic autoimmune disorders. The other autoimmune disorders were detected in 9 of 19 (47 %) asymptomatic pts and 7 of 62 (11 %) symptomatic patients ($p < 0.05$).

Conclusion: Twenty-three percent of pts with AIH were asymptomatic during diagnosis, most of them had other autoimmune disorders. Forty one percent of pts with AIH presented with acute onset. This value was higher than the literature and it could be related as our clinic tertiary center with liver transplant unit. Nineteen percent

of patients with cirrhosis reflects the insidious progression of autoimmune hepatitis.

P-0221

Clinical characteristics in acute presentation of autoimmune hepatitis

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Background/aim: Although autoimmune hepatitis (AIH) was usually categorized to chronic active hepatitis, some patients develop the disease with acute presentation (AP). The aim of current study was to clarify the clinical differences in AIH with AP from classical AIH.

Methods: Retrospective investigation was performed for AP among 153 patients with AIH, histologically diagnosed between 1995 and 2014. AP was defined to be a case with higher level in total bilirubin over 5 mg/dl and/or in ALT than 400 U/L. AIH cases with moderate or more liver fibrosis and no AP development were given as control. **Results:** A total of 68 AP patients (56 females, 56 median y-o) were enrolled. AP patients were younger than control (median 56 vs. 64 y-o, $P < 0.05$). Compared with control, AP cases showed lower albumin level (3.8 vs. 3.4 mg/dl, $p < 0.05$) and increased PT INR (1.23 vs. 1.07, $p < 0.05$) despite of higher platelet counts ($p < 0.05$). Disease deterioration (PT % < 60) was more frequent in AP than in control (23/69 vs. 2/32, $P < 0.01$). Although there were no significant difference in serum IgG levels and ANA titer between AP and control group, Patients with lower titration in ANA than 40× were 37 % in AP, but 14 % in control. Hepatocyte collapse in zone 3 was found 57 % in AP, and 38 % in classical AIH. There was no difference in revised AIH score between them.

Conclusion: From higher risk of disease deterioration, AIH with AP could be distinct from classical one.

P-0222

Is there a link between autoimmune hepatitis and bronchial asthma and irritable bowel syndrome?

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Background and aims: Autoimmune hepatitis (AIH) is characterized by chronic inflammation of the liver/interface hepatitis/hypergammaglobulinemia/presence of autoantibodies. And the idea of an involvement of autoimmunity in the pathogenesis of bronchial asthma (BA) has been proposed by many studies. Besides, some studies suggested that irritable bowel syndrome (IBS) and dysmotility to some extent might be of an autoimmune origin. But there has been no report for the relationship between AIH, BA and IBS. Hence we investigated the prevalence of these categories and the comorbidity to suggest the possible relationship .

Methods: Total 3564 patients with AIH or BA or IBS at the single tertiary hospital between January 2002 and December 2014 were reviewed retrospectively. We included histopathologically and serologically proven AIH, BA with positive methacholine bronchial provocation test, and IBS fulfilling Rome III criteria. We analysed the base characteristics of each category and the prevalence of the comorbidity.

Results: Among total patients, 2259 cases fulfilled inclusion criteria (Table 1). The number of the diagnosis of AIH, BA, IBS was 108, 1243, 913 respectively. And all comorbidity cases were 5. The case with AIH and BA was 1, and 1 with AIH and IBS as well, and 3 with BA and IBS.

Conclusions: The prevalence of the comorbidity of AIH and BA or IBS was very rare, even in BA with IBS. These results suggest that the immunologic pathogenesis of AIH, BA and IBS might be quite different. Further studies are necessary to investigate the factors which are coexistent or distinct between AIH and other autoimmune diseases.

Is there a link between autoimmune hepatitis and bronchial asthma and irritable bowel syndrome?

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Table 1. Patients characteristics and comorbidity

	AIH	BA	IBS
No. of cases	108	1243	913
Age at the diagnosis mean ± SD (range)	48.2 ± 15.5 (16–80)	36.7 ± 28.1 (1–95)	48.5 ± 14.2 (10–93)
Gender M:F (%)	14:94 (13:87)	606:637 (49:51)	424:489 (46:54)
	AIH+BA	AIH+IBS	BA+IBS
No. of cases	1	1	3
Age at the diagnosis, mean	AIH: 40 BA: 37	AIH: 46 IBS: 42	BA: 32.3 IBS: 33
Gender M:F	0:1	1:0	2:1

P-0223

Aortic aneurysm and aortic dissection in patients with autoimmune hepatitis: a report of four cases

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Cardio-vascular diseases (CVDs) are rare in autoimmune hepatitis (AIH) patients. We report here four cases of AIH. In three of them aortic aneurysm developed and in the other case acute aortic dissection happened. In aneurysm cases, two showed abdominal aortic aneurysm (AAA), and the other one showed both AAA and aortic arch aneurysm. All four cases were female, and diagnosis of AIH was made at the age between 56 and 68. All cases fulfilled the Interna-

tional Diagnostic Criteria of AIH scoring more than 15 (1999 version), or more than 7 (2008 version) before treatment and were diagnosed as definite AIH. Oral steroid therapy was mostly effective, but minor relapses were sometimes observed. The interval of diagnosis between AIH and CVD was 8, 9, 10 and 23 years. At the diagnosis of CVD, their liver condition was chronic hepatitis except one cirrhotic case, who was also associated with hepatocellular carcinoma (HCC). None of them had diabetes, hyperlipidemia or family history of CVDs. None of them were drinker. Two patients were smoker and had hypertension. In the case with HCC, chest X ray study showed abnormal shadow of vascular disease, angiography showed winding abdominal artery trunk, and autopsy revealed abdominal aortic aneurysm, suggesting that she had Takayasu's aortitis. Takayasu's aortitis is known to be associated with HLA haplotype of A24-B52-DR2(DRB1*1502), and the case with HCC had also this particular haplotype. Further studies will be needed to elucidate whether AIH would have any relation to aortitis or other CVDs.

P-0224

Successful treatment with steroid of autoimmune hepatitis in a patient with rectum adenocarcinoma

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Introduction: Autoimmune hepatitis (AIH) is very rarely seen after diagnosis and treatment of neoplastic diseases. Eight cases, including seven hematological malignancy and one colon cancer was reported in literature, so far. There is limited information regarding these patients. Here, we report a case of AIH with liver metastasis from rectum adenocarcinoma.

Case: A 65 year-old woman was diagnosed with rectum adenocarcinoma four years ago and treated with colectomy and chemotherapy. Three years after the diagnosis of cancer, she was admitted with jaundice to Oncology Department. Liver ultrasonography and tomography showed a 4 × 3.5 cm well defined heterogeneous enhancement solid focal lesion in segment 4A. Three months after liver metastasectomy, liver tests were elevated. Anti-nuclear antibodies and anti-smooth muscle antibodies at a titer of 1:3200 were positive. The non-neoplastic liver tissues from metastasectomy was re-examined by a pathologist and pathologic findings, including porto-portal bridging, rare nodule formation and plasma cells infiltration were observed. In light of this laboratory and pathological findings, we diagnosed autoimmune hepatitis and started glucocorticoid monotherapy. Calcium and vitamin D3 combination therapy for osteoporosis prophylaxis was also given. At the end of 10th month, liver tests were normal and neoplastic lesions in liver and colon were no detected in radiologic imaging.

Conclusion: AIH is rarely occur in individuals with neoplastic diseases. The neoplastic disease itself may affect the immune regulatory mechanisms as triggering factor. In treatment of these patients, steroid monotherapy can be used safely, but these patients should be closely monitored for recurrence of neoplastic disease or AIH.

P-0225

Hepatocellular carcinoma in autoimmune hepatitis: the Western India experience

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Backgrounds and aims: Autoimmune hepatitis (AIH) can progress to cirrhosis and primary hepatocellular carcinoma (HCC) is thus an anticipated, though unusual, consequence. The aim of this study was to determine the incidence of HCC in AIH, to identify the potential risk factors and to find out whether AIH also belongs to a high risk group that mandates surveillance.

Methods: Medical records of 242 patients diagnosed with AIH were analyzed retrospectively. Among the patients who developed HCC, demographic, clinical, biochemical, endoscopy, imaging and treatment details were analyzed.

Results: 242 patients were diagnosed with AIH (149 females; 93 males). 174 patients (72 %) had evidence of cirrhosis of liver (100 females, 74 males). Median age at diagnosis of AIH was 44.65 ± 1.59 years with a total follow-up of 798.75 patient years. Commonest presenting symptom was jaundice (49.17 %) followed by ascites (34.29 %), hepatic encephalopathy (0.02 %) and gastrointestinal bleed (0.016 %). Eight patients (3 %) were identified with HCC (5 females; 3 males). The incidence of HCC equated to 1001.56 cases per 100,000 patient follow-up years. Median age at diagnosis of HCC was 58.62 ± 1.03 years. Risk factors noticed in patients developing HCC in AIH were cirrhosis of liver, BMI above 25, age above 52 years, thrombocytopenia and presence of esophageal varices.

Conclusion: AIH with cirrhosis of liver predisposes to HCC. Incidence of HCC in AIH is lower than chronic liver disease secondary to viral hepatitis B and C, alcohol or NASH. Routine screening and surveillance for early detection of HCC must be performed in all AIH patients developing cirrhosis. Further prospective studies are needed to validate this data.

P-0226

A case of PJP diagnosed during treatment with 15 mg prednisolone daily for AIH

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Background: Clinical practice guidelines for autoimmune hepatitis (AIH) edited by the research team of the Ministry of Health, Labour and Welfare in Japan are unclear about the optimal times for starting and stopping sulfamethoxazole/trimethoprim treatment in patients receiving prednisolone. We here present an illustrative case and discuss preventive administration of sulfamethoxazole/trimethoprim.

Case report: A 71-year-old woman was referred to our department because of impaired liver function. In accordance with the revised criteria for diagnosing AIH of the International Autoimmune Hepatitis Group, a diagnosis of “probable AIH” was made. She began

steroid medication with 60 mg prednisolone daily; the dosage being gradually reduced thereafter. More than 2 months after starting steroid medication, when her dosage was 15 mg daily, she developed *Pneumocystis jiroveci* pneumonia (PJP), which was diagnosed by polymerase chain reaction on a sample of bronchoalveolar lavage. Treatment with sulfamethoxazole/trimethoprim was successful.

Discussion: Patients with autoimmune inflammatory disorders who develop PJP reportedly have CD4⁺ counts of <250 cells/mm³, six of eight cases (75 %) in one series having counts <200 cells/mm³ (*Chest* 2000;118:712–720). Sowden et al. recommend performing CD4⁺ counts after 1 month’s immunosuppressive therapy only in patients who satisfy the following three criteria: (1) steroid dosage >15 mg prednisolone or equivalent/day; (2) >3 months corticosteroid treatment planned; and (3) total lymphocyte count <600 cells/mm³ (*BMC Infect Dis* 2004;4:42). Lower CD4⁺ counts may warrant use of prophylactic sulfamethoxazole/trimethoprim. Our patient’s CD4⁺ count was 175 cells/mm³ when she was receiving 15 mg prednisolone daily.

P-0227

Two cases of hepatocellular carcinoma with autoimmune hepatitis

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Introduction: HCC due to AIH was rare but recently has increased by the progression of the therapy for AIH. We reported here two cases of hepatocellular carcinoma (HCC) with autoimmune hepatitis (AIH) and investigated relationship between HCC and AIH.

Case description: Case 1: The patient was 66-year-old female with AIH for 21 years. She had been treated for AIH with PSL and azathioprine. The tumor was 2.6 cm in diameter, and involved moderately differentiated HCC. The non cancerous lesion showed liver cirrhosis. Case 2: The patient was 79-year-old female with AIH for 10 years. She had been treated for AIH with PSL. The tumor was 2.8 cm in diameter, and histologically involved moderately differentiated HCC. The non cancerous lesion showed liver cirrhosis. In these case, AIH were well-controlled and HCC was developed by considerably later after initiation of therapy for AIH. Therefore, careful follow-up is necessary for diagnosis of HCC caused by AIH.

P-0228

Usefulness of elastography for diagnosis of liver fibrosis and disease progression in PBC patients

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Background and aims: Assessment of biliary fibrosis in primary biliary cirrhosis (PBC) is needed to evaluate disease progression and the effectiveness of therapies. Our aim was to first evaluate the diagnostic performance of real-time tissue elastography (RTE) and vibration controlled transient elastography (VCTE), and then to assess

elasticity of the liver and spleen as predictive markers for progression of symptomatic PBC (s-PBC).

Methods: All participants provided written informed consent, and the study protocols were approved by the institutional ethics committee. Forty-five patients with PBC diagnosed by laparoscopic liver biopsy were enrolled. We measured liver and spleen elasticity by RTE and VCTE. Symptoms caused by PBC were also evaluated. From these examinations, we first conducted prospective performance analysis of RTE and VCTE for the diagnosis of METAVIR fibrosis stages by using receiver operating characteristic (ROC) curve analysis. Second, 36 patients with asymptomatic PBC (a-PBC) were followed up regularly every 1–3 months.

Results: RTE and VCTE showed high performance and were significantly superior to biochemical markers in diagnosing significant fibrosis, severe fibrosis, or cirrhosis. During the follow-up period, 10 patients (27.7 %) developed liver-related symptoms. Putative prognostic factors were studied using uni- and multivariate analyses. Multivariate analysis showed that splenic elasticity by RTE represents an independent factor for development of liver-related symptoms (HR: 1.4; 95 % CI: 1.1–1.5; $P < 0.001$).

Conclusions: Splenic elasticity determined with RTE is the most closely associated parameter for evaluating liver-related symptoms and is useful as a predictive marker of symptom development in patients with a-PBC.

P-0229

A family with four siblings affected with primary biliary cirrhosis

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PBC is a chronic autoimmune cholestatic liver disease which predominantly affects middle-aged women. Genetic factors play an important role in PBC susceptibility. The contribution is evidenced by twin studies and the familial clusters. Epidemiological studies have demonstrated that first degree relatives of PBC patients are at higher risk of developing PBC. A 56-year-old woman was referred to our hospital with abnormal liver function test. Her past history was unremarkable, no history of alcohol or drug abuse. Laboratory findings revealed an elevated AST 127 IU/L, ALT 160 IU/L, ALP 132 IU/L, GGT 169 IU/L, and AMA was positive. Liver biopsy was done and non-suppurative destructive cholangitis and granulomatous inflammation with fibrosis was noted. The patient was diagnosed with PBC and her family members, including four sisters and three brothers were evaluated. One of the sisters was born of a different mother. She was not diagnosed with PBC. The other four full sisters' (including the target patient) serum AMA was positive and elevations of serum AST, ALT, ALP, and GGT were noted. Liver biopsy was done and the results were compatible with PBC. Characteristics and initial laboratory findings of the patients are described in Table 1. Patient 3 was initially presented with acute hepatitis-like features. The patients were treated with UDCA and liver biochemistry results showed good response. The patients are being followed with UDCA therapy without complications.

Conclusions: Evaluation of the family members of PBC patients are necessary even if they are asymptomatic, for early diagnosis and treatment leading better prognosis.

P-0230

Up-regulated miR-139-5p in hepatocytes induce TNF α similar to clinical sera from PBC cases

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Background: We previously reported that microRNA profiles in serum among early and advanced PBC. miR-139-5p had specific pattern in advanced PBC. Accordingly, we evaluated the function of this miRNA to clarify the mechanism in the disease progression.

Methods: Huh 7, and T lymphoma cell line, Jurkat, were evaluated. We have artificially either up or down regulated miR-139-5p in both cell lines by a lipofection method. Firstly, we disseminated 70 % confluent cells to six well plates. After one passed overnight, we up-regulated by addition of miRNA mimic. Conversely, we down-regulated using miRNA inhibitor. After transfection, we cultured in 2 or 3 days, and subsequently harvested cell culture supernatant. Finally, we measured various pro-inflammatory cytokines' concentrations by the ELISA.

Results: Among some cytokines in Huh7 after 3 days culture, TNF α was significantly elevated (average: 385.7 pg/mL) compared with that of controls (33.2 pg/ml). However, TNF α in that of Huh7 with down-regulated was not different to control's. In addition, TNF α of up-regulated miRNA after 2 days culture was 51.4 pg/mL. Therefore, the TNF α increased over time. On the contrary, either up or down introduction of miR139-5p in T cell line did not changed the levels of TNF α .

Conclusions: Our findings are accordance with the previous report describing TNF α in the sera of advanced PBC. Thus, up-regulated miR-139-5p in hepatocytes could results in the increase TNF α in portal area, which in turn induce exacerbation of injuries cholangiocytes. Our findings suggest that PBC hepatocytes might have a possibility of important background cell which induce progression.

P-0231

Laparoscopic finding can predict the progression in patients with primary biliary cirrhosis

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Background: Regarding the progression of primary biliary cirrhosis (PBC), at least two different types of progression in PBC have been proposed; one is a hepatic failure-type progression, and the other is a portal hypertension-type progression. Laparoscopy has a great advantage in that it enables observation of the entire surface of the liver and facilitates obtaining a biopsy at the focal site of diseases. In this study, we assessed laparoscopic findings to predict the rapid progression to liver failure in patients with asymptomatic PBC at the time of diagnosis.

Methods: At Our Hospital 194 patients were diagnosed as PBC from 1980 to 2014. Of those, 160 patients who had asymptomatic PBC was used for further analysis. The importance of laparoscopic findings was evaluated for disease progression in comparison with biochemical data, histological classifications, and K-7 expression in hepatocyte.

Results: During the follow-up period, nine patients developed jaundice, and the median period until the development of jaundice was 5.8 years (1.6–12.6 years). In proportional hazards analysis, age less than 55 years old ($p = 0.0379$, HR 2.03×10^9), ALP higher than 360 IU/L ($p = 0.0009$, HR 41.27), high K-7 expression in hepatocyte ($p = 0.0002$, HR 274.53), No reddish patch in laparoscopic finding ($p = 0.0017$, HR 82.78), yellowish or whitish liver color ($p = 0.0094$, HR 12.45) were the predictive factors of the development of jaundice.

Conclusion: Because bile duct lesions are heterogeneous even in one PBC patient's liver, laparoscopic liver biopsy is useful to predict liver failure in patients with asymptomatic PBC.

P-0232

Quantitative fibrosis parameters highly predict esophageal varices of primary biliary cirrhosis

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Background and aims: Esophageal varices (EV) may develop in any histological stages of primary biliary cirrhosis (PBC). We aimed to validate quantitative fibrosis (qfibrosis) parameters in portal, septal and fibrilla area as ideal predictors of EV, as well as to evaluate effectiveness of recent criteria for EV screening.

Methods: We retrospectively enrolled PBC patients who had undergone liver biopsy, second harmonic generation (SHG)/two-photon excited fluorescence (TPEF) microscopy imaging and upper esophagogastrosocopy. Parameters in portal, septal and fibrilla area were assessed by multivariate analysis.

Results: After screening 112 PBC patients, a total of 19 patients were included in this study. 52.63 % (10/19) of the patients had EV; the percent of EV in early stage and late stage are 37.50 % (3/8) and 77.78 % (7/9) respectively. The qfibrosis parameters of number of thick/thin/short/long strings and distributed collagen percentage in septal area, distributed collagen percentage in fibrillar area were significantly different in PBC patients with EV and without EV. The number of thick strings in septal area was an independent factor to predict EV (odds ratio, 1.6; 95 % confidence interval, 1.019–2.511), with 70.00 % sensitivity and 88.89 % specificity. Comparison of different EV screening criteria showed the number of thick strings in septal area had the highest diagnostic performance of specificity, negative predictive value, positive predictive value and accuracy.

Conclusions: Number of thick strings in septa area as an independent risk factor can highly predict EV in PBC patients. Further validation is required.

Keywords: Quantitative fibrosis, Primary biliary cirrhosis, Esophageal varices.

P-0233

Relationship between serum cytokeratin-18 (M30) and primary biliary cirrhosis pathology

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Objective: Cytokeratin-18 (CK-18) is a marker of cellular apoptosis and necrosis, and is known to be increased in patients with non-alcoholic steatohepatitis (NASH). The relationship between primary biliary cirrhosis (PBC) pathology and CK-18 levels is not fully understood. Accordingly, this study aimed to examine the relationship between serum CK-18 levels and PBC pathology.

Methods: Subjects were 35 patients with PBC who were diagnosed at our hospital or affiliated facilities. Serum CK-18 levels were measured by ELISA, and correlations with laboratory test values of patients with PBC were assessed. Relationships with liver histological findings (Nakamura classification) were also assessed.

Results: Patients with PBC had the following background characteristics: mean age, 58 ± 11 years; male:female ratio, 7:28; ALT, 57 ± 40 U/L; ALP, 544 ± 354 U/L; IgM, 491 ± 341 mg/dl; presence/absence of chronic non-suppurative destructive cholangitis (CNSDC), 17/18; and Nakamura classification: CA (0/1/2/3), 10/3/5/17; HA (0/1/2/3): 10/9/14/2; stage (1/2/3/4): 6/16/10/3. Serum CK-18 levels (U/l) were as follows (healthy individuals/NASH/CHC/PBC): $370 \pm 185/458 \pm 181/432 \pm 147/572 \pm 265$, with significantly higher levels found in patients with PBC compared to healthy individuals ($p < 0.01$). Correlations between serum CK-18 levels and laboratory test values were as follows: ALT ($r = 0.2038$, $p = 0.2477$); ALP ($r = 0.03699$, $p = 0.8355$); and IgM ($r = 0.5685$, $p < 0.0005$), with IgM showing a positive correlation. Liver histology and serum CK-18 levels (U/l) were related as follows: presence/absence of CNSDC, $457 \pm 231/694 \pm 249$ ($p < 0.01$); CA (0–1/2–3), $445 \pm 266/647 \pm 240$ ($p < 0.05$); HA (0–1/2–3), $487 \pm 246/674 \pm 258$ ($p < 0.05$); and Stage (1/2–4), $277 \pm 181/621 \pm 240$ ($p < 0.005$), with higher CK-18 levels observed with disease progression.

Conclusion: CK-18 levels and PBC pathology were closely related.

P-0234

Serum cell death biomarkers for prediction of liver fibrosis and poor prognosis in PBC

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The development of simple, noninvasive markers of liver fibrosis is urgently needed for primary biliary cirrhosis (PBC). This study examined the ability of several serum biomarkers of cell death to estimate fibrosis and prognosis in PBC. A cohort of 130 patients with biopsy proven PBC and 90 healthy subjects were enrolled. We assessed the utility of the M30 ELISA, which detects caspase-cleaved cytokeratin-18 (CK-18) fragments and is representative of apoptotic cell death, as well as the M65 and newly developed M65 Epideath

(M65ED) ELISAs, which detect total CK-18 as indicators of overall cell death, in predicting clinically relevant fibrosis stage. All three cell death biomarkers were significantly higher in patients with PBC than in healthy controls and were significantly correlated with fibrosis stage. The areas under the receiver operating characteristic curve for the M65 and M65ED assays for differentiation among significant fibrosis, severe fibrosis, and cirrhosis were 0.66 and 0.76, 0.66 and 0.73, and 0.74 and 0.82, respectively. In multivariate analysis, high M65ED (hazard ratio 6.13; 95 % confidence interval 1.18–31.69; $P = 0.031$) and severe fibrosis (hazard ratio 7.45; 95 % confidence interval 1.82–30.51; $P = 0.005$) were independently associated with liver-related death, transplantation, or decompensation. High serum M65ED was also significantly associated with poor outcome in PBC (log-rank test; $P = 0.001$). Noninvasive cell death biomarkers appear to be clinically useful in predicting fibrosis in PBC. Moreover, the M65ED assay may represent a new surrogate marker of adverse disease outcome.

P-0235

Risk-factors for mortality in PBC patients: a deceased case-living control study in China

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Background: Primary biliary cirrhosis (PBC) is a slowly progressive autoimmune liver disease, which may result in liver failure and death. This study was designed to investigate the risk factors for liver-related mortality in PBC patients.

Methods: The data of all deceased PBC patients were collected from Beijing 302 Hospital database, which contains 1255 PBC inpatients during the period from 2002 to 2013. The controls were matched to cases by gender, age (± 2 years) and date of hospital admission (± 6 months). Potential risk factors were included for evaluation, and odds ratios (OR) and 95 % CI were estimated using univariate (unadjusted OR) and multivariate (adjusted OR) conditional logistic regression. Cutoff value of risk factor was determined by receiver operator characteristics analysis.

Results: Based on the data of 126 liver-related deceased PBC cases and 504 controls, we found that the initial diagnostic stage of the disease, high level of total bilirubin (TBIL), low levels of albumin (ALB) and platelet (PLT), non-response to ursodeoxycholic acid (UDCA) and diabetes mellitus were independently associated with significant increase in the risk of liver-related mortality in PBC patients ($P < 0.001$). Cutoff values of TBIL and ALB for prediction of poor prognosis were determined as 34.5 g/L and 37.65 $\mu\text{mol/L}$, the area under the receiver operating characteristic curve was 0.759 and 0.770 ($P < 0.001$).

Conclusions: This study indicates that levels of ALB, TBIL and PLT at the initial diagnosis of disease, non-response to UDCA and diabetes mellitus were all independent risk factors for liver-related mortality in PBC patients.

P-0236

PD-1 as an “immune-check disturbance” with infiltrated CD8⁺CXCR3⁺ T cells modify PBC clinical course

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Primary biliary cirrhosis (PBC) is an autoimmune liver disease characterized by a cholangitis of small bile ducts. CD8⁺T cell priming on the enterohepatic circulation is believed to lead to PBC. We aim to investigate the role of inhibitory receptor PD-1 associated with CD8⁺CXCR3⁺T cells in PBC/PBC-AIH patients, demonstrating the “immune-check disturbance” mechanism.

Methods: A total of 20 liver tissue specimens were collected from our department, including ten PBC patients, eight from diagnosed patients with PBC-AIH and two from patients with chronic virus hepatitis (CVH). The clinical data of the patients were collected. We examined immune-histochemical expression of PD-1 in the liver tissue and CD8⁺CXCR3⁺ cells using double immune-fluorescence method.

Results: Enlarged lymph nodes in peritoneal cavity (LA) were observed in PBC-AIH patients. Pathologically, massive lymphocyte infiltration in the liver could be observed in LA group. Accordingly, most CXCR3 positive cells were CD8 positive. Infiltration of CD8⁺CXCR3⁺ cells in the interface or around small bile ducts was significantly more extensive in LA patients with PBC-AIH than CVH and non-lymph node enlargement group (NLA). Expression of PD-1 in the liver tissue was significantly lower in the LA group than the NLA and CVH group. There was a negative correlation between the PD-1 expression and liver function results with elevated CD8⁺CXCR3⁺ T cells.

Conclusions: PBC patients with lymphadenopathy progressively showed more serious liver injury. Our research could provide novel evidence that intra-abdominal infection factor circulation could be an etiology and promoter of PBC aggregation, which will provide new sight for individual treatment algorithm.

P-0237

Clinical significance of the serum IgG3 in primary biliary cirrhosis

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Background and aims: Some primary biliary cirrhosis (PBC) patients have a high serum IgG3 level, but its clinical significance is unclear. This study was undertaken to clarify the clinical characteristics of PBC patients with high and normal serum IgG3 levels.

Methods: Fifteen PBC patients were divided into a group of eight patients with normal serum IgG3 levels (normal-sIgG3 group) and a group of seven patients with high serum IgG3 levels (> 89 mg/dl, high-sIgG3 group), and their clinical, serological, and pathological characteristics were retrospectively compared.

Results: (1) The proportion of patients with an associated autoimmune disease, including systemic sclerosis and CREST syndrome, in the high-sIgG3 group (50 %, 4/8) was higher than in the normal-sIgG3 group (14 %, 1/7), and the serum IgA level of the high-sIgG3 group was significantly higher than in the normal-sIgG3 group (377 vs. 195 mg/dl, $p = 0.003$). (2) Based on the Scheuer’s classification (stages 1/2/3/4) of the patients whose liver tissue could be evaluated the histological findings in the high-sIgG3 group (0/4/0/1) were similar to those in the normal-sIgG3 group (2/2/1/0). However, two

patients in the high-sIgG3 group alone had developed liver cirrhosis clinically. (3) The serum ALP and GGT levels were higher in the high-sIgG3 group than in the normal-sIgG3 group.

Conclusion: The PBC patients with high serum IgG3 levels tended to have an associated autoimmune disease, high serum IgA levels, and high biliary enzymes values. We speculate that the serum IgG3 level reflects the disease activity of PBC.

P-0238

Red cell distribution width is related to histologic severity of primary biliary cirrhosis

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Backgrounds/aims: We aimed to investigate whether red cell distribution width (RDW) and RDW-to-platelet ration (RPR) are related to histologic severity of primary biliary cirrhosis (PBC).

Methods: Seventy-three patients who had undergone liver biopsy and first diagnosed as PBC followed between January 2010 and January 2015 were enrolled in our research. Histological staging is based on Ludwig's and Scheuer's classifications. The patients were divided into early-stage (Stage I) and late-stage (Stage II, III, IV). All common demographics, clinical characteristics, hematological parameters, liver biochemistry and AMA-M2 were retrospectively analyzed. RPR, APRI, FIB-4 were calculated.

Results: A total of 28 patients with early-stage group (38.4 %), whereas 45 had late-stage (62.6 %). Patients with late-stage had significantly higher RDW (13.6 vs 14.4, $p = 0.019$), conjugated bilirubin (10.1 vs 23.4, $p = 0.029$) and significantly lower cholinesterase (7901.1 vs 6060.8, $p = 0.001$), platelet (212.6 vs 167.0, $p = 0.006$). However, there were no differences between groups in terms of other routine parameters which had been previously used in PBC, like AST, MPV. The sensitivity and specificity of RDW were 33.3 and 92.9 %, area under the receiver-operating characteristic curve (AUROC) is 0.66. The sensitivity and specificity of RPR were 46.7 and 96.4 %, AUROC is 0.74 ($P < 0.001$). Compared with pre-existing indicators, RPR showed a higher AUROC than APRI (0.648, $P = 0.035$) and FIB-4 (0.682, $P = 0.009$).

Conclusion: RDW and RPR may be a new non-invasive marker for predicting histologic severity of PBC.

P-0239

Autoimmune hepatitis (AIH) in patients transferred from pediatric to adult hepatology care

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Background: Autoimmune hepatitis is chronic liver disease that require long term treatment and follow-up.

Aim: This is a retrospective analysis of patients with childhood AIH onset who were transferred from pediatric to adult hepatology care. The aim of the study was to assess patient's status at the age of 18 years.

Material and methods: 110 patients (M:34; F:76) with AIH diagnosed between 1990 and 2005 at age 6–17 (mean \pm SD 11.7 \pm 2.5) who were treated at pediatric center for 1–12 years (6.3 \pm 2.5) until adulthood were analyzed.

Results: Marked reduction of mean values of laboratory parameters between disease presentation and last pediatric control were noted: ALT activity from 492 \pm 421 to 57 \pm 45 U/l, IgG concentration from 3241 \pm 1263 to 1468 \pm 478 mg/dl, gammaglobulins from 35.3 \pm 12.0 to 17.1 \pm 4.8 g/l and bilirubin from 3.2 \pm 2.8 to 0.07 \pm 4.8 mg/dl. However high rate of patients remained with abnormal laboratory results: ALAT-50 %, IgG-38 %, gammaglobulins-56 %. Liver histology (Batts–Ludwig score) improved respectively: grading from 2.7 \pm 0.8 to 1.0 \pm 0.9 and staging from 2.6 \pm 0.9 to 1.8 \pm 1.0, but 67 % of patients had features of liver inflammation and 91 % developed liver fibrosis. At the last control at pediatric site 51 % of patients were on combined steroid-azathioprine therapy, 12 % were on steroid monotherapy, 14 % azathioprine monotherapy and 24 % of patients did not receive any immunosuppressive treatment. Eight patients presented portal hypertension and seven patients had marked osteoporosis.

Conclusion: AIH therapy at pediatric center improved mean values laboratory parameters and histology however the rate of patients with abnormal results remains high and most of patients require treatment.

P-0240

Possible involvement of activating follicular helper T cells in autoimmune hepatitis

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Background: There is an increasing interest in the role of follicular helper T (T_{fh}) cells in autoimmunity. Dysregulated T_{fh} cells have been shown to be responsible for the induction of fatal autoimmune hepatitis (AIH) in mice with disruption of programmed cell death 1 (PD-1) after neonatal thymectomy. This study is aimed to reveal the involvement of T_{fh} cells in the immune pathogenesis of human AIH. **Methods:** Heparinized peripheral blood was collected from 28 patients with AIH and 38 healthy volunteers (HC). After mononuclear cells were separated, various surface markers were then investigated using flow cytometry. Cytokine production was investigated after stimulation with PMA and ionomycin. We also investigated the distribution of CXCR5⁺ CD4⁺ cells in the liver using immunohistochemical staining.

Results: CXCR5⁺ CD4⁺ T_{fh} cells comprised 8.5 % (median) (range 2.7–18.3), and 8.1 % (3.1–13.5) of the total T cells in the blood of patients with AIH and HC, respectively. The T_{fh} cells were then classified into several subsets according to their expression of PD-1, inducible co-stimulator (ICOS). We found that the frequency of ICOS⁺ T_{fh} cells was significantly decreased in the PSL-treated patients (median 22.6 %, range 15.2–34.5) compared with the patients who were not treated with PSL (median 35.7 %, range 26.0–56.2). CXCR5⁺ cells were detected among infiltrated lymphocytes in the portal area of the liver of patients with AIH. Furthermore, we confirmed that T_{fh} cells secreted IL-21 and IL-17 and IFN γ after stimulation.

Conclusions: Our results suggest that ICOS⁺ Tfh cells may be associated with the disease activity of AIH.

P-0241

Long term follow up of AIH children after 6–12 months course of budesonide and azathioprine therapy

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Background: Budesonide in combination with azathioprine was tested as a treatment option in patients with AIH between 2003 and 2009 (BUC-38-AIH).

Aim: The aim of this study is to present the data of AIH patients switched from budesonide to standard of care treatment.

Methods and patients: This is retrospective analysis of 15 patients (M-3, F-12) with AIH diagnosed at the age 7–16 (mean \pm SD 11.2 ± 2.8) years who participated in BUC-38-AIH study and received 6–12 months course of combined budesonide and azathioprine therapy. After completion of the study patients continued standard of care AIH treatment for 1.5–6 years (3.5 ± 1.7) until they reached 18–19 years of age and were transferred for further care to adult hepatology clinic. Liver function tests, IgG, gammaglobulin, liver biopsy results and treatment at the final visit at paediatric site were analysed.

Results: Laboratory results elevated at the beginning of budesonide trial were markedly reduced at the end of budesonide treatment and remained stable at the final paediatric visit respectively: ALT 395 ± 387 ; 61 ± 99 and 49 ± 44 U/l; gamma-globulin 24.6 ± 6.7 ; 16.1 ± 2.0 and 16.9 ± 6.0 g/l and IgG: 2388 ± 715 ; 1606 ± 265 and 1642 ± 330 mg/dl. Grading of inflammation in liver biopsy improved from 2.5 ± 0.92 before budesonide treatment to 0.86 ± 0.7 at the end of paediatric observation and staging respectively from 2.0 ± 0.85 to 1.3 ± 1.0 . At the final visit at paediatric site seven patients continued steroids and azathioprine, six patients received azathioprine monotherapy and two patients were off medication.

Conclusions: Patients who completed budesonide therapy remained stable until the end of observation in paediatric site.

P-0242

Evaluation of factors associated with relapse of type 1 AIH patients

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Background: Although the long-term prognosis of autoimmune hepatitis (AIH) patients is good, repeated relapses during treatment

increase risk of liver cirrhosis or cancer. However, risk factors of relapse are unknown. This study investigated factors associated with AIH relapse.

Methods: We collected data for 65 patients' diagnosed with type 1 AIH from 2009 to 2014 at four major hospitals in our area, and retrospectively assessed their clinical and laboratory data. We analyzed differences in characteristics between patients with and without relapse after remission by treatment. Relapse was defined based on International Autoimmune Hepatitis Group (IAIHG) criteria.

Results: Relapse occurred in 8 (12.5 %) patients. Initial daily dose of prednisolone (PSL) in relapsed patients was 30–40 mg. After tapering, PSL dose was maintained at 2–10 mg daily until relapse, except one case relapsed after PSL withdrawal. Age at diagnosis was significantly younger in patients with relapse compared to without relapse (51.3 ± 13.5 vs 63.7 ± 11.4 ; $P < 0.01$). There were no differences in serum ALT, IgG levels, antinuclear antibody positive rates, disease severity, and revised IAIHG scoring between groups. Positive rates of anti-smooth muscle antibody (ASMA) were significantly higher in patients with relapse compared to without relapse (80.0 ± 26.5 %; $P = 0.02$).

Conclusions: ASMA might be a useful marker to predict relapse of type 1 AIH, although a larger or prospective study is required to confirm these results.

P-0243

Liver transplantation in primary biliary cirrhosis: a retrospective cohort study

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Aims: To assess liver transplantation (LTX) treatment effect in PBC.

Methods: This is a retrospective observational study including 55 consecutive patients treated with LTX for PBC between January 1, 2006 and August 31, 2014 and performed uni- and multivariate analysis for morbidity and mortality, in particular to define the influence of median model for end-stage liver disease (MELD) to these parameters. The severity of disease was quantified using MELD scores.

Results: Fifty five patients receiving LT [mean (SD) age 50 (9) years, 86 % female] were included. The mean duration of follow-up after LTX treatment was 43.0 months. The mean MELD score of these 55 patients was 16.2. The average Mayo score was 8.5. The median interval from diagnosis to LTX was 64.6 months. 82 % of patients was treated with UDCA (first 45 %, second 85 %). The mean duration of UDCA treatment for the entire period analyzed was 30.2 months. The indication for LTX was liver failure in 35 (63 %) cases (Fig. 1). 1 (1 %) patient underwent liver transplantation for the presence of hepatocellular carcinoma. The complications of LTX were infections in 34 (61 %) patients, biliary complications in 18 (32.7 %) patients, portal vein thrombosis in 3 (5 %) patients, de novo HBV infection in 2 (3.6 %). Recurrence of primary biliary cirrhosis was demonstrated by histologic findings in 5 (9 %) patients. Three patients died from multiple organ failure, infection and intraperitoneal hemorrhage respectively.

Conclusion: Orthotopic liver transplantation is the only effective curative therapy for end-stage PBC. PBC patients should receive prophylactic HBIG infection to prevent de novo HBV infection.

P-0244

A case of autoimmune hepatitis successfully treated with ursodeoxycholic acid

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A 42-year old woman was diagnosed with autoimmune hepatitis without histological examination in the other hospital in 2007. She was prescribed ursodeoxycholic acid (UDCA) 600 mg/day, and her liver function was normalized. In 2011 she consulted our hospital for moving. She continued internal use of UDCA, and her liver function maintained normal. However, she stopped UDCA internal use without having a medical examination after having received a prescription in February, 2014. Because she had abnormal liver function test pointed out by the periodic medical examination that she underwent in the other hospital in December, 2014, she consulted our hospital again. We restarted internal use of UDCA and performed liver biopsy in January, 2015, and she had a diagnosis of autoimmune hepatitis histologically. Her liver function is normalized and maintains normal. The standard treatment of autoimmune hepatitis is corticosteroid, but there are some reports about the effect of UDCA. Because the safety of UDCA is established, a utility of UDCA is expected in the case that degree of hepatitis is mild, and in the case who had diabetes that side effects of the corticosteroid are concerned. We report the case that liver function was exacerbated by internal use interruption of UDCA, but was able to maintain normalization of the liver function by a repeated dose of UDCA.

P-0245

An older patient with autoimmune hepatitis, which drugs-induced liver injury probably may trigger

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A 70-year-old Japanese male, who has taken omeprazole, bisoprolol fumarate, camostat mesilate, kallidinogenase, powdered senna leaf, and benzbromarone, felt fatigue. Benzbromarone and the others began to be taken in March 2012, and more than one year before, respectively. His liver dysfunction was pointed out at November, 2012. Two months later, he was treated with ursodeoxycholic acid and branched chain amino acid. However, his symptom was not improved, and he was introduced and admitted to our hospital June, 2013. The edema was observed in his lower legs. Blood examination data showed as following: AST 45 U/L, ALT 18 U/L, T-Bil 1.9 mg/dl, albumin 2.8 g/dl, PT 69 %, IgG 2636 mg/ml, and ANA 80-fold. Examination for infection showed past infection of HBV. Abdominal ultrasound showed mild ascites. We initially suspected drug-induced liver injury and stopped all pills. Then diuretic was started, and PT % and albumin levels were slightly improved. But IgG level remained high, so liver biopsy was performed. Histological findings of liver showed chronic hepatitis (F2 A3), also indicating autoimmune hepatitis compatible. We diagnosed him as autoimmune hepatitis, triggering probably with drug-induced liver injury. After prednisolone 15 mg daily was started, abnormal liver dysfunction was improved

and his fatigue was also disappeared. Prednisolone was tapered to 5 mg daily and kept at this dose, and no exacerbation has been observed. Although it is famous that benzbromarone has liver-toxicities, we diagnosed him as autoimmune hepatitis with liver biopsy.

P-0246

The association between non-alcoholic steatohepatitis and intrahepatic cholangiocarcinoma

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Background: This study aimed to investigate the association between non-alcoholic steatohepatitis (NASH) and intrahepatic cholangiocarcinoma (ICC).

Methods: This was a hospital based case control study of patients who underwent surgical resection either for ICC or for metastatic liver tumor (the control group). We assessed their clinical characteristics, pathological findings, and the prevalence of known ICC risk factors such as hepatitis B or C infection, hepatolithiasis, alcohol abuse, or a history of exposure to chlorinated organic solvents. For patients without these risk factors (34 patients in the ICC group and 69 patients in the control group), we compared other factors including the prevalence of NASH diagnosed by pathological examination.

Results: In the patients without known risk factors, 15 patients in the ICC group and 13 patients in the control group were diagnosed with NASH. Univariate analysis showed significantly higher values in the ICC group for the mean age of patients ($P = 0.0478$), prevalence of obesity ($P = 0.0365$), prevalence of NASH ($P = 0.0078$), and serum levels of albumin and gamma-glutamyl transpeptidase (g-GTP) ($P = 0.0051$ and $P = 0.0006$, respectively) compared with the control group. Multivariate analysis showed that age and serum levels of g-GTP and NASH were independent risk factors for ICC. In patients with NASH, the proportion of patients with hepatic fibrosis was significantly higher in the ICC group than in the control group ($P = 0.0014$).

Conclusion: NASH is a possible risk factor for ICC development. The hepatic fibrosis caused by NASH may be important in the development of ICC.

P-0247

A case of mucinous carcinoma of the liver

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Mucinous carcinoma of the liver (MCL) has been classified as special types of intrahepatic cholangiocellular carcinoma. And MCL remains one of the most severe neoplastic disease. A case is 70 years old female. A subtotal thyroidectomy was performed in July 2011 in the diagnosis of papillary thyroid cancer. A contrast enhanced abdominal CT scan showed low density tumor with enhancement in the periphery and calcification in the center at the lateral lobe of the liver

during a post-operatively follow up. Laboratory data revealed almost normal ranges. CEA and CA19-9 levels are high. Hepatitis B surface antigen and hepatitis C virus antibody were negative. With a preoperative diagnosis of intrahepatic cholangiocellular carcinoma or liver metastasis of papillary thyroid cancer, a left lobectomy of the liver was carried out. Pathological findings showed adenocarcinoma produced a large amount of mucin, with mucus lake. And signet-ring cells detected in the mucus lake. The pathological diagnosis was MCL (T2 N1 M0 Stage IVB). Chemotherapy (S-1 and Gemcitabine) and hyperthermia was started after operation. At present, 3 years since the surgery, the patient remains well with lymph node metastasis. MCL is an extremely rare intrahepatic cholangiocellular carcinoma. We report our case, along with the relevant literature.

P-0248

Vorinostat-incorporated nanoparticles for local delivery into human cholangiocarcinoma cells

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Background: This study was to evaluate the anticancer activity of vorinostat and vorinostat-incorporated nanoparticles (vorinostat-NPs) against HuCC-T1 human cholangiocarcinoma cells. Vorinostat-NPs were fabricated by a nanoprecipitation method using poly(DL-lactide-co-glycolide)/poly(ethylene glycol) copolymer.

Results: Vorinostat-NPs exhibited spherical shapes with sizes less than 100 nm. Vorinostat-NPs have anticancer activity similar to that of vorinostat in vitro. Both vorinostat and vorinostat-NPs have equal potency in terms of inhibition of histone deacetylase expression, mutant type p53 suppression, increased p21 expression and PARP/cleaved caspase-3 expression. However, vorinostat-NPs showed improved antitumor activity against HuCC-T1 cancer cell-bearing mice compared to vorinostat, whereas empty nanoparticles had no effect on tumor growth. Furthermore, vorinostat-NPs increased the expression of acetylated histone H3 in tumor tissue and suppressed HDAC expression in vivo. The improved anticancer activity of vorinostat-NPs can be explained by molecular imaging studies using near-infrared (NIR) dye-incorporated nanoparticles, i.e. NIR-dye-incorporated nanoparticles were intensively accumulated in the tumor region rather than normal one.

Conclusions: Our results demonstrate that vorinostat and vorinostat-NPs exert anticancer activity against HuCC-T1 cholangiocarcinoma cells by specific inhibition of HDAC expression. Thus, we suggest that vorinostat-NPs are a promising candidate for anticancer chemotherapy in cholangiocarcinoma.

P-0249

Intrahepatic cholangiocarcinoma arising in patients with hepatocellular carcinoma

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Background: Intrahepatic cholangiocarcinoma (ICC) sometimes arises in patients with hepatocellular carcinoma (HCC) synchronously or during the clinical course of HCC. We investigated the clinical features of patients with HCC and ICC.

Methods: We enrolled patients with pathologically diagnosed HCC and ICC by searching our patient database. Clinical characteristics of the patients were retrieved by reviewing medical records.

Results: Among 3820 HCC patients treated in our department between January 1992 and December 2013, seven patients (M/F = 6/1) were identified who met the criteria. The median age at the time of diagnosis of ICC was 69 years. Six patients had hepatitis C virus infection and one had alcoholic liver disease. ICC was first recognized by dynamic CT or MRI in all patients, with imaging features of peripheral enhancement in the early and late phases. One patient had synchronous HCC and ICC, and underwent liver resection. Six patients were diagnosed ICC during recurrent HCC surveillance. HCCs had been treated with radiofrequency ablation, transarterial chemoembolization or hepatic resection. ICCs were treated with radiofrequency ablation, hepatic infusion chemotherapy, or systemic chemotherapy. All except one patients could not undergo standard chemotherapy due to impaired liver function. Five patients died of cancer progression, one patient liver failure and one patient ischemic heart disease. The median survival time after diagnosis of ICC was 285 days.

Conclusions: ICCs were first suspected with atypical findings on imaging. The prognosis after ICC was poor due to aggressive tumor characteristics and impaired liver function.

P-0250

A case of intraductal papillary neoplasm of the bile duct associated with autoimmune hepatitis

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Intraductal papillary neoplasm of the bile duct (IPNB) is a cystic tumor of the liver defined separately from hepatic mucinous cystic neoplasm. IPNB shows a papillary intraductal growth with frequent communication to neighboring bile duct, and recent studies have suggested that IPNB is a biliary counterpart of intraductal papillary mucinous neoplasm of the pancreas. Development of liver cancer in patients with autoimmune hepatitis (AIH) reported previously have been hepatocellular carcinoma (HCC), but an increased incidence of malignant tumor other than HCC in AIH is expected because recent advances in treatment regimens based on steroid or azathioprine have achieved a high degree of efficacy. We report a 50-year-old man with IPNB associated with AIH who underwent a successful extended left hepatectomy. The resected specimen was a cyst-forming tumor. Immunohistochemical staining of the tumor cells demonstrated negativity for MUC1, MUC2, CK20, CDX2, CD56, chromogranin A, synaptophysin, estrogen receptor and progesterone receptor, and positivity for CK7, CK19, MUC5AC, MUC6, p53, Ki-67, CEA and CA19-9. It was compatible with a pancreatobiliary-type IPNB accompanied by carcinoma in situ. The histopathology of the liver showed an interface hepatitis with plasma cell infiltration, which was diagnosed as AIH. The patient has remained free of cancer recurrence following the hepatectomy, and liver dysfunction caused by AIH has been controllable by steroid administration for more than 15 years.

P-0251

A case of asymptomatic mucinous cystic neoplasm of the liver with marked calcification

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Background: Mucinous cystic neoplasms (MCN) of the liver are defined as cyst-forming neoplasms, showing no communication with the bile ducts, composed of cuboidal to columnar mucin-producing epithelium, associated with ovarian-type stroma in WHO 2010. MCNs are rare and nearly always symptomatic, and calcification of the cyst wall is occasionally seen. Here, we report a case of asymptomatic MCN with marked calcification.

Case: 44-year-old woman was pointed out a liver cystic mass by medical checkup and referred to our hospital for further examination. Computed tomography revealed a multilocular cystic mass of 8-cm in diameter in the right liver. The walls were thick with marked calcification and showed enhancement by contrast material. On magnetic resonance cholangiopancreatography, communication between the cystic mass and the bile duct was not detected. At first, cystic echinococcosis was suspected because she has a history of residence in Pakistan and India. But a serum echinococcosis antibody test was negative. She underwent surgery because possibility of a cystic neoplasm was not denied.

Result: We performed right hepatectomy. The postoperative course was uneventful and she was discharged at 6 days after the surgery. Gross-section of the resected specimen showed mucus or necrotic tissue in the cysts. Microscopically, mucus-producing epithelium was lined into the cyst wall and ovarian-like stroma was observed. There was no component of invasive carcinoma. Final diagnosed MCN.

Conclusion: MCN is a differential diagnosis of asymptomatic multilocular cystic mass with marked calcification of the wall.

P-0252

A rare case: an elderly women with type 3 choledochal cyst

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Introduction: Choledochal cysts are rare congenital anomalies. It is seen in approximately 1 per 100,000 live births. Approximately % 60 of them were diagnosed in the first year of life. Because of the risk of developing malignancy, the treatment of these cysts are usually surgery. Here, we present our experience with tip 3 choledochal cyst in an elderly patient.

Case: 92-year-old female patient was admitted to our gastroenterology clinic with complaints of abdominal pain, fever, and jaundice. Cholestatic enzymes elevation and leukocytosis were detected in her laboratory tests. As dilatation of the bile ducts was observed in

hepatobiliary ultrasonography, acute cholangitis was diagnosed in this patient. In her medical history, she was also describe some findings compatible with two times cholangitis in the last 4 months. It was decided to perform ERCP for the treatment. ERCP was failed due to periampullary duodenal diverticulum and precut sphincterotomy was performed. With cessation of oral intake, hydration and intravenous antibiotics the patient was improved. When we performed EUS and MRCP for cholangitis etiology; type 3 bile duct cysts was observed. ERCP was performed again and sphincterotomy was done after the successful choledoch cannulation was achieved. The patient was discharged after recovering completely.

Conclusion: Bile duct cysts are rare pathologies and diagnosed mostly in the first year of life. Nevertheless; even if the patient is elderly choledochal cyst should be kept in mind in etiology of recurrent cholangitis. Though the treatment of these cysts are usually surgery; ERCP and sphincterotomy is sufficient for type 3.

P-0253

Male patient with anaphylactic shock and hepatic cyst

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Male patient with anaphylactic shock and hepatic cyst MA. 36 years old male patient with unremarkable medical history presented to the ER with sudden onset of decreased level of consciousness associated with sweating and abdominal pain. O/E there was evident of hypotensive patient with cold extremities and rapid pulse with diffuse abdominal tenderness but with no signs of peritoneal involvement. Laboratory data was unremarkable apart from leukocytosis with predominant neutrophilia. Following urgent resuscitation with iv fluid and iv corticosteroid the patient recovered and he was admitted in the Medical Department for further evaluation. Abdominal US was positive for hepatic big cystic lesion measuring 10 cm diameter raising suspicion of huge hepatic hydatid cyst. Ct abdomen with contrast was arranged and imaging (showed below) confirm the diagnosis of huge hepatic hydatid cyst. Ct chest and brain were normal with no evidence of cystic lesions involvement. Pertinent patients history revealed that he lived most of his life in rural area in Syria and there was repeated contact with domestic dogs and cats. The patient was commenced on albendazole and urgent surgical consultation with experienced biliary and hepatic surgery doctor was arranged who recommend surgical resection over Percutaneous aspiration which was done and the patient was discharged home after 7 days of uneventfully postoperative care. Final diagnosis: huge hepatic hydatid cyst

P-0254

Clinical evaluation of 40 cases with treated hepatic cysts

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Background: Patients with symptomatic, infectious or hemorrhagic hepatic cysts need medical treatment, although most patients with hepatic cysts are asymptomatic.

Methods: Forty cases with hepatic cysts, treated from 1998 to 2014, were evaluated their clinical characteristics, efficacies and outcomes according to each therapeutic methods. When reduction of the treated cyst was confirmed by image examination 1 month after treatment or later, the treatment was defined as “successful”.

Results: Treated patients were mainly middle-aged females and abdominal pain and distension were the most common symptoms. Among 40 cases, 10 cases had simple cysts (SC) and 17 cases were diagnosed as polycystic liver disease (PLCD). Percutaneous aspiration therapy was performed in 13 cases and 4 cases obtained successful effect. Percutaneous sclerotherapy was performed in 20 cases using minocycline or ethanol as sclerosing agents. Thirteen cases obtained successful effect and ethanol were more effective as therapeutic agent than minocycline. Sclerotherapy brought higher therapeutic effect than aspiration therapy in cases with SC, while there was no significant difference of effect between the two therapies in cases with PLCD. Fenestration was performed in 5 cases, and 3 cases of them obtained successful effect. Hepatic resection was performed in 1 case, whose cystic lesion was not able to deny a malignancy. We did not experience severe complications with Clavien–Dindo Classification Grade IIIb or greater in the total 49 therapeutic procedures.

Conclusions: Percutaneous sclerotherapy may be primary treatment for symptomatic hepatic simple cysts in view of efficacy and safety.

P-0255

Expression of PRK/PLK3 is associated with a less invasive tumor biology in cholangiocarcinoma

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Cholangiocarcinoma (CCA) cells in vivo express tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) rendering these cells dependent on survival pathways to circumvent TRAIL induced cell death. The hedgehog pathway exerts survival signals by interaction with cell cycle regulating polo-like kinases (PLK). In contrast to PLK1/2, proliferation-related kinase PRK/PLK3 was associated with improved clinical outcome of CCA-patients. Here we aimed to characterize the role of PRK for CCA tumor biology. We employed control and siPRK-KMCH-1 as well as HUCCT-1 CCA cells for these studies. Fibroblast growth factor (FGF) can induce PRK expression and is present in CCA as examined by qRT-PCR. rhFGF increased PRK in CCA cells within 2 h and PRK returned to basal levels after 6 h. This effect appears to be mediated by the FGF signal transducer phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K) as FGF-induced PRK upregulation was blocked by the PI3K inhibitor LY294002. As strong PRK expression was shown to be associated with reduced lymph/blood vessel infiltration in CCA-patients resulting in improved overall survival rates, we investigated the effect of PRK silencing on CCA cell migration. Indeed, knockdown of PRK increased tumor cell migration in a manner that was neither confounded by proliferation effects nor mediated by nuclear factor-kappa B (NF-κB) signaling or increased epithelial mesenchymal transition. These results suggest strong PRK expression as a positive predictor of a favorable clinical outcome in CCA-patients due to a less invasive tumor cell behavior. Therefore, approaches employing PLK-inhibitors

for the treatment of human CCA should comprise targeting PLK1 and PLK2 but not PRK/PLK3.

P-0256

Photosensitizer-incorporated chitosan nanoparticles for treatment of cholangiocarcinoma

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Water-soluble chitosan (WSC) is derived from chitosan and has low molecular weight. Since chitosan is only soluble in acidic water, WSC has advantages such as excellent solubility in deionized water, ease of chemical modification, altered functionality and enhanced bioactivity. Especially, WSC is regarded to have enhanced anti-carcinogenicity, anti-microbial activity, and, especially, enhanced mucosal delivery of bioactive agents. In this study, we prepared photosensitizer-incorporated WSC nanoparticles through ion complex formation between WSC and photosensitizer, Ce6. Since WSC has cationic properties, ion complexes can be formed with anionic drug, DNA and photosensitizer. Photosensitizer-incorporated WSC (ChitoCe6) has small particle sizes less than 100 nm at TEM observation and particle size measurement. They enhances Ce6 delivery to HuCC-T1 human cholangiocarcinoma cells rather than Ce6 itself. Furthermore, Ce6-incorporated WSC nanoparticles generated ROS higher than Ce6 itself in spite of anti-oxidant properties of WSC. Ce6-incorporated WSC nanoparticles enhances phototoxicity against tumor cells and induced apoptosis/necrosis of tumor cells. We suggest that WSC nanoparticles is simple and promising vehicles for photosensitizer delivery.

P-0257

ERCP role in management of biliary strictures in Southern Egypt

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Background: Biliary stricture is a challenging condition that needs multiple tools for management. ERCP is a valuable procedure in managing obstructive biliary diseases.

Aim of the work: Evaluate ERCP role in management of biliary strictures in our locality.

Patients and methods: This prospective study included 100 patients with biliary strictures induced cholestasis to admitted to endoscopy unit, Qena University Hospital from January 2013 to June 2015. Patients subjected to full clinical, laboratory and imaging studies. Patients were divided into group 1 included 56 patients (32 males, 24 females) with malignant strictures and group 2 included 44 patients (15 males, 29 females) with benign strictures. For all patients ERCP was done.

Results: The age ranged from 35 to 83 years in group 1 and from 17 to 65 years in group 2. Successful cannulation achieved in 89.2 % of group 1 patients and in 93.2 % of group 2 patients. Group 1 biliary strictures were distal in 64 %, mid in 14 % and proximal in 22 % of patients. Group 2 biliary strictures were distal in 41 %, mid in 32 %, proximal in 22 % and multiple in 5 % of patients. Malignant strictures were due to cancer pancreas in 60 %, cholangiocarcinoma in 20 % and other malignancies in 20 % of patients. Benign strictures were iatrogenic (post-cholecystectomy) in 44 % and pancreatitis induced in 24 % of patient. Successful biliary drainage was achieved in 89.2 % of group 1 patients by stents fixation and in 93.2 % of group 2 patients (sphincterotomy 16 % and stents fixation in 77.2 %). Complications occurred in 3.3 % of all patients.

Conclusions: ERCP is an effective tool for management of biliary strictures.

P-0258

Safety of conscious sedation in patients undergoing ERCP with hepatobiliary disease

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Background: Endoscopic retrograde cholangiography is a technically demanding but highly important modality to diagnose and treat hepatopancreatobiliary disorders; intravenous midazolam, nalbuphine and propofol are used to perform ERCP. Aim of our study was to determine the safety of conscious sedation in patients undergoing ERCP for hepatobiliary disease.

Methods: A retrospective study was conducted in Liaquat National Hospital, Karachi, who underwent ERCP during 2010–2015.

Results: A total of 413 procedures were performed, total male were 144 (34.8 %). The mean age was 50.16 ± 15.45 years. Conscious sedation was used in 326 patients (78.9 %). In five patients (1.59 %) procedures were converted from conscious sedation into general anesthesia because patients were restless despite of maximum sedation. Mean dosages of Inj. midazolam, nalbuphine per each patient were 6 ± 2 , 7 ± 3 , mg. respectively. There is overall success rate in 319 (98 %) in conscious sedation. Choledocholithiasis were present in 199 patients (61.04 %). CBD strictures were present in 68 cases (28.8 %) of cases. biliary leak seen in 12 cases (3.6 %). CBD worms (1.2 %). Pediatric cases were excluded.

Conclusion: This current study documents that ERCP procedures are safe under conscious sedation as it provides reasonable sedation even in elderly patients, minimal side effects, cost savings and useful in patients in whom general anesthesia is contraindicated, propofol administration requires more intensive monitoring and assistance of anesthesiologists.

P-0259

Outcomes of endoscopic retrograde cholangiopancreatography in patients with cirrhosis

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Background: ERCP is commonly performed in cirrhotics, they are at increased risk of bleeding after ERCP, but the incidence and risk-

factors in this population is not well described. The aim of this study was to examine the incidence of post-ERCP complications.

Methods: We evaluated all ERCP's performed in cirrhotics at our practice from 2008 to 2014. We analyzed a matched control group of non-cirrhotic with ERCP during same period. Patient and procedure-related risk factors were analyzed. Predictive factors for post-ERCP complications were determined by univariate and multivariate analysis.

Results: 200 ERCP's were analyzed (100 in cirrhotic and 100 in non-cirrhotic patients). Complications occurred in 14 patients (14 %) in the cirrhosis group (11-mild, 3-severe): pancreatitis 3 cases (3 %), cholangitis 3 cases (3 %), and post-sphincterotomy bleeding 6 cases (6 %). The overall rate of complications were significantly higher in patients with cirrhosis (14 vs 6.4 %, $P = 0.04$). Bleeding occurred more commonly in patients with cirrhosis compared to non-cirrhotics [(7.0 %) vs (1.7 %), $P = 0.03$]. The incidence of pancreatitis and cholangitis were not different in both groups. Logistic regression identified sphincterotomy [OR 8.99 (CI 1.08–74.60), $P = 0.04$] and cirrhosis [OR 3.51 (CI 1.11–11.09), $P = 0.03$] as independent risk factors of post-ERCP complications, whereas cirrhosis was the only independent risk factor of post sphincterotomy bleeding [OR 4.12 (CI 1–16.93), $P = 0.04$].

Conclusions: The rate of complications after ERCP in patients with cirrhosis (14 %) is higher than that of non-cirrhotics. Cirrhosis and sphincterotomy are risk factors of post ERCP complications.

P-0260

Role of fluorescence in situ hybridization in the diagnosis of malignant biliary stricture

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Introduction: A wide spectrum of hepatobiliary and pancreatic diseases, both benign and malignant, can result in the development of biliary strictures. Although trans abdominal ultrasound, computed tomography, and magnetic resonance imaging are sensitive for detecting bile duct pathology, they do not reliably distinguish strictures as being malignant or benign. FISH utilizes fluorescently labeled DNA probes to chromosomal centromeres or unique loci to detect cells that have numerical or structural abnormalities indicative of malignancy.

Aim of the study: To evaluate the accuracy of FISH in the diagnosis of malignant biliary stricture.

Patient and methods: Forty eight patients with indeterminate bile duct stricture presented with obstructive jaundice were prospectively enrolled and underwent ERCP and FISH over a period of 20 month from June 2013 to February 2015. Four patients were excluded because of loss follow-up. Thus, 44 patients were included in data analysis (17 males and 27 females).

Results: The final diagnosis of stricture type was 32 malignant and 12 benign cases. FISH was positive in 30 cases and negative in 14 cases; sensitivity, specificity, positive predictive value, negative predictive value, and accuracy were 93.75, 100.00, 100.0, 85.7, 95.5 and 0.969 respectively.

Conclusion: FISH increase the diagnostic accuracy of malignant biliary stricture. The sensitivity of FISH could improve the clinical management of patients being evaluated for malignant bile duct

stricture by enabling a definitive diagnosis at an earlier stage in the clinical evaluation.

Keywords: Indeterminate bile duct stricture, MRI, ERCP, FISH.

P-0261

Efficacy of minor endoscopic sphincterotomy to prevent post-ERCP pancreatitis for biliary stenting

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Objectives: Endoscopic sphincterotomy (ES) is performed to reduce the risk of post-ERCP pancreatitis (PEP) and facilitate the stent placement. But there is still debate the necessity of ES due to potential complications like bleeding. In this study, we evaluated efficacy and safety of minor ES for self-expandable metal stent (SEMS) placement. **Methods:** From December 2008 to December 2014, we reviewed 244 consecutive patients with malignant biliary obstruction, diagnosed as unresectable malignancies who received SEMSs with minor ES, retrospectively. Early post-ERCP complications including such as PEP and bleeding rate were evaluated.

Results: There were 244 subjects with 118 patients from cholangiocarcinoma, 79 patients from pancreatic cancer, 47 patients from non-pancreaticobiliary malignancy. Overall early post-ERCP complications occurred among ten patients (4.1 %), including PEP in seven patients (2.9 %, 6 mild and 1 moderate), mild bleeding in two patient (0.8 %), and mild cholangitis in one patient (0.4 %). No significant difference in cancer type (cholangiocarcinoma versus pancreatic cancer versus others, $p = 0.696$) and SEMS type (uncovered versus covered, $p = 1.000$) were noted in frequency of post-ERCP complications. Comparison with no complication group ($n = 234$) and complication group ($n = 10$), statistically significant differences were only found in the number of inserted SEMS ($p = 0.014$)

Conclusion: The strategy of minor ES could be feasible, safe and effective to facilitate the SEMS placement as not increasing severe bleeding or other complications in patients with malignant biliary obstruction.

P-0262

Gender specific etiological spectrum of ERCP in hepatopancreaticobiliary diseases

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Background: Aim of our study was to determine the spectrum of gender specific hepatobiliary disorders on basis of ERCP findings.

Methods: A retrospective review of ERCPs procedure were done among patients with hepatobiliary disease from April 2010 to 2015 conducted in Liaquat National Hospital, Karachi.

Results: Total of 416 patients, the mean age was 50.16 ± 15.45 years, Majority of patients were female 269 (64.6 %). Biliary cannulation was successful in 404 cases 97.11 % of cases and most common diagnosis in female gender includes choledocholithiasis 186 cases (69.1 %) followed by biliary strictures seen in 53 cases

(19.70 %). Most common diagnosis in male includes choledocholithiasis in 82 cases (55.7 %) followed by biliary strictures in 41 cases (27.89). The association of gender and diagnosis was found significant with $p = 0.003$.

Conclusion: This current study reveal that most notable biliary pathology is choledocholithiasis affecting female gender in majority cases. Biliary strictures were seen more commonly in male gender.

Keywords: CBD stones, Biliary stricture, Endoscopic retrograde cholangiopancreatography.

P-0263

Relationship between adipocytokine levels and post-ERCP pancreatitis risk

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Introduction: Individuals with increased visceral adiposity have a systemic inflammatory microenvironment. Therefore, these individuals are considered to be more sensitive and more prone to severe acute pancreatitis. Adipocytokines that markedly effect the course of pancreatitis can also contribute to the development of post ERCP pancreatitis (PEP). We aimed to investigate the relationship between adipocytokine levels and PEP risk.

Methods: Eighty two patients with diagnosis of choledocholithiasis and 30 healthy controls were enrolled in this prospective study. Pre-procedural chemerin, vaspin, IL-6 and PEP risk factors were compared between PEP and non-PEP groups.

Results: Mean age was 56.3 ± 14.4 years and 51 patients were female. In patient group, adipocytokine levels, body mass indexes and waist circumferences were higher than controls. Total cannulation success and mean procedure time were 82.9 % and 28.7 ± 8.8 min. PEP developed in 12 (14.6 %) patients. Chemerin levels in PEP group was higher than non-PEP group (580.2 ± 172.5 vs. 392.2 ng/ml ± 168.2 , $p < 0.01$). Insulin resistance was higher in PEP group than non-PEP group ($p: 0.001$). But there was no statistically significant difference between PEP and non-PEP groups in terms of pre-procedural vaspin, TNF alpha, IL-6, CRP levels. According to logistic regression analysis, increased chemerin levels, HOMA- >2.5 and pancreatic duct cannulation were found to be independent risk factors for PEP (OR: 1.006, $p: 0.006$, OR: 4.57, $p: 0.05$, OR: 6.54, $p: 0.02$).

Conclusion: Elevated serum chemerin levels and insulin resistance are independent risk factors for the development of PEP.

P-0264

Role of Vit K in prevention of post sphincterotomy bleeding in patients with normal prothrombin time

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The importance of intravenous vitamin K administration for patients with obstructive jaundice and prolonged PT who will undergo ERCP

to prevent PSB cannot be overemphasized. However, its use in cases wherein PT is within normal range has limited data.

Aim: To determine the association of giving pre-procedural intravenous vitamin K and identify clinicopathologic factors in patients with normal PT on the rate of PSB.

Methods: Consecutive patients with normal PT (INR \leq 1.3) prior to ERCP were included. Presence or absence of PSB was recorded and correlated with patient's clinicodemographic profile and vitamin K administration. Factors with p value \leq 0.1 on bivariate analysis were entered into logistic regression analysis to determine independent predictors for PSB.

Results: Sixty two (62) patients with normal prothrombin time were included. On bivariate analysis, vitamin K administration is not a significant predictor of PSB (p value = 0.420); whereas gender (p value = 0.038), previous hepatobiliary surgery (p value = 0.078) and serum creatinine levels (p value = 0.01) were identified as predictors for PSB. On logistic regression analysis, there was a trend for increased bleeding in males [adjusted OR = 2.57 (95 % CI 0.46, 14.52)] and those with previous hepatobiliary surgery [adjusted OR = 2.85 (95 % CI 0.50, 16.11)] but did not reach statistical significance (p value = 0.285, 0.237 respectively).

Conclusion: pre-procedural vitamin K administration is not a significant predictor of PSB in patients with normal prothrombin time who will undergo ERCP. A trend for increased bleeding for males and those with previous hepatobiliary surgery was noted but did not reach statistical significance.

P-0265

Cooling via a gallbladder drainage tube during radiofrequency ablation reduces gallbladder injuries

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Background: Liver tumors within 1 cm away from gallbladder were risky for radiofrequency ablation (RFA) due to potential thermal injuries to the gallbladder. The purpose of this study is to establish a new approach to protect gallbladder from thermal injuries by setting up a cooling system into the gallbladder with an in-vivo porcine model.

Methods: Six male pigs were used in this study. The study group comprised three pigs which had received endoscopic gallbladder drainage (EGBD) under general anesthesia. The control group had three pigs without receiving EGBD. An internally cooled monopolar radiofrequency electrode with a 2-cm electrically active tip was percutaneously deployed parallel to the long axis of gallbladder and 0.5 cm distant from the gallbladder wall. RFA was performed for 6 min in all pigs. Chilled water had been continuously perfused via EGBD tube throughout RFA in the study group. All pigs were sacrificed and examined immediately after ablation.

Results: All of the pigs without protection by the EGBD cooling system had discoloration of the gallbladders adjacent to the ablated liver. Moreover, two of them had gallbladder perforation and

sloughing of gallbladder mucosa. None of the pigs protected by EGBD cooling system had significant thermal injuries. One received EGBD had minimal catheter tip related damage to the gallbladder mucosa.

Conclusions: Chilled water perfusion through EGBD was a feasible method to minimize RFA related thermal injuries to the gallbladder.

P-0266

Endoscopic management of occluded biliary stents: a single center study

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Background and aim: Self-expandable metal stents (MSs) and plastic stents (PSs) are widely used for malignant hilar biliary obstruction (MHBO). Recent progress in chemotherapy has prolonged the survival of patients with MHBO, leading to increased stent occlusion rates. This study investigated the survival durations and patency durations after the first and re-interventions.

Patient and methods: Sixty-eight patients who underwent transpapillary biliary MS and/or PS placement for MHBO were retrospectively analyzed. Hilar biliary obstruction was due to cholangiocarcinoma in 57 cases and gallbladder carcinoma in 11 cases. The stent type, stent patency, and patient survival were investigated. Statistical tests were two-sided; $p < 0.05$ was considered statistically significant.

Results: MSs, PSs, and MSs and PSs were placed in 38, 25, and 5 patients, respectively. Bilateral stenting was performed in 22 patients. The rate of receiving chemotherapy was 36.8 %. Median patency of the stent showed significant difference between the MS (234 days) and PS (96 days) groups, but showed no significant difference between the bilateral and unilateral stenting groups. Median patient survival showed significant difference between the groups with and without chemotherapy. Nineteen patients had undergone stent occlusion. The re-intervention rate was 84.2 %. And the rate of receiving chemotherapy was 10.6 %. Median patency of the secondary stent showed no significant difference between the MS groups (10 cases) and PS groups (6 cases).

Conclusion: MSs were more effective for drainage of MHBO. However, no significant differences were observed for the patency durations of the secondary stent between MS and PS groups.

P-0267

Psoriasis and neutrophilic cholangitis: more than a coincidence

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Neutrophilic cholangitis (NC) is a recently described entity characterized by neutrophilic infiltration of biliary tree causing cholestasis. Although it has been reported in few cases of psoriasis, its prevalence

may be high. We report a case presented with recurrent psoriatic arthritis and skin plaques and with cholestasis confirmed to be NC. A 39-year-old male was admitted with joint pain, skin plaques, and fever. He has been diagnosed psoriasis 20 years ago and was treated with local steroid. On this admission, he had fever (39 C, tympanic), pain on right upper abdominal region, pain on axial and peripheral joints, disseminated psoriatic rashes, a sausage-shaped digit (left hand, third finger), pustules on psoriatic rashes on both ankles, and pitting on all hand nails. He had right-sided sacroiliitis on MRI. Blood culture was obtained and he was given antibiotics. Fever, rash, and joint pain persisted; blood cultures remained sterile. A liver biopsy revealed neutrophilic cholangitis. Antibiotics were discontinued. He was given ursodeoxycholic acid, pulse steroid, then methylprednisolone 20 mg/day. Body temperature returned to normal after first dose of steroid and the rash evanesced. Right upper abdominal pain improved. Methotrexate was added to the therapy and steroid was tapered. ALT, AST, bilirubins and alkaline phosphatase returned to normal. NC appears as an extradermal manifestation of psoriasis. There are six case reports and a study searching for prevalence of NC among 22 psoriatic patients. These recent data suggest that it has been underestimated and physicians should be aware of this involvement in psoriasis patients.

P-0268

Management of biliary complications post liver transplant in Singapore

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Introduction: Biliary complications (BC) are common causes of morbidity following liver transplantation (LT). Long term outcome of BC post LT in Singapore is unknown. We aim to describe the outcome of BC following LT.

Methods: Patients with LT performed from 2005–2015 were prospectively monitored for BC. BCs were diagnosed using magnetic resonance cholangiopancreatography, endoscopic retrograde cholangiography (ERC) or T-tube cholangiography. Total follow up duration was 4086 months. Kaplan Meier curves were used for survival analysis. Statistical analysis was performed with SPSS 21.0.

Results: Total of 81 LT patients were recruited. Median age was 61.5 years and 69.1 % were male. Percentages of disease donor liver transplant (DDLTL) versus living donor liver transplant (LDLT) were 71.6 vs 28.4 % respectively. BC developed in 30.9 % of LT patients (21 strictures, 7 leaks and 2 choledocholithiasis and 1 Sphincter of Oddi dysfunction). BC have worse 5-year survival (84 vs 58 %, $p = 0.013$). Among patient with biliary stricture, 81 % had anastomotic stricture (AS) and 19 % had non-anastomotic stricture (NAS). NAS had worse survival than AS ($p < 0.001$). AS were treated with ERC and stenting with 57.1 % achieve complete resolution after an average of 5 ERC over median intervention period of 16.8 months. Complications of ERC were low [cholangitis (1.33 %) and bleeding (1.33 %)]. LDLT has more BC (56.5 vs. 22.4 %, $p = 0.004$) than DDLT.

Conclusion: BC led to worse outcome among LT patients. LDLT suffered more BC including biliary strictures and leak. NAS was associated with poorer prognosis than AS. ERC is a safe and effective treatment option for AS.

P-0269

Multi-planar or curved-planar reconstruction in the detailed CT analysis of cholelithiasis

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Introduction: Cholelithiasis is a common disease. However, it is sometimes accompanied by other serious diseases. Therefore, we must carefully interpret the CT images of other biliary systems, such as cystic duct and extrahepatic bile duct. Recent development of computer technology has enabled us to make many axial CT images into a single image, such as multi-planar or curved-planar reconstruction (MPR or CPR), rapidly. We report two interesting cases of cholelithiasis combined with early bile duct cancer or gallbladder volvulus which were diagnosed by MPR or CPR without contrast medium.

Cases 1: A 53-years old man was admitted to our hospital for the examination of mild elevations of the liver enzymes. MDCT depicted a gallbladder stone (3 mm). More detailed analysis using MPR images showed a protruded lesion (3×10 mm) in middle-inferior part of bile duct. Pylorus preserving pancreatoduodenectomy was performed. Histologically, the tumor was a papillary adenocarcinoma within the fibrous muscular layer without lymph node metastasis.

Case 2: A 79-year-old woman presented with abdominal pain without a fever. MDCT revealed thickened gallbladder wall, and a gallstone (10 mm). More detailed analysis using CPR images revealed a string-like cystic duct and a V-shaped distortion of the extrahepatic bile duct. The patient was diagnosed to have gallbladder torsion. Emergency operation was performed, and we confirmed gallbladder torsion.

Conclusion: MPR or CPR images provide important information for diagnosis of the small protrusion of early bile duct cancer and the twisted narrow structure of the cystic duct.

P-0270

Functional role of microRNAs-FoxA2 axis in cholestatic liver injury

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Background and aims: The biliary tree is a complex network of interconnected ducts that increase in diameter from small to large bile ducts formed small cholangiocytes (SMCCs) and large cholangiocytes (LGCCs). microRNAs and the definitive endoderm marker, FoxA2, are the key factors that regulates cell differentiation and tissue regeneration. Our aim was to characterize the functional role of microRNAs-FoxA2 axis in biliary progenitor cells during cholestatic liver injury.

Methods: Bile duct ligation (BDL) and MDR2 knockout mice (MDR2^{-/-}) were used as animal models of cholestatic liver injury with healthy transplanted cholangiocytes in NOD/SCID mice.

Results: Silenced let-7b and miR-200b, along with activated FoxA2 expression were observed in murine small bile ducts in BDL and MDR2-/- mice liver, suggesting that they are the important mediators for biliary remodeling. FoxA2 has been demonstrated to be the direct target of let-7b and miR-200b in biliary epithelial cells by luciferase reporter assay. We examined the benefits of serum chemistry from transplanted cells. Serum ALT and AST levels in NOD/SCID mice engrafted with SMCCs (3X107, i.p.) showed significant changes compared with vehicle treated mice (n = 5), along with the significantly improved sirius red staining. Reduced let-7b and miR-200b levels, along with the enhanced expression of definitive endoderm differentiation marker FoxA2 and reduced biliary fibrosis was observed in BDL mice liver after SMCC cell therapy.

Conclusion: The therapeutic effect of biliary-committed progenitor cells during cholestatic liver injury is mediated by let-7b/miR-200b-FoxA2 axis, the known critical regulators of biliary development and injury.

P-0271

Helicobacter pylori in pancreatic adenocarcinoma

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Background: Pancreas cancer is one of the leading causes of pancreas related deaths. It is important to know the risk factors. Epidemiological studies have shown that *Helicobacter pylori* (*H. pylori*) could be a risk factor in pancreas cancer but the studies to establish this are ongoing.

Aim: To investigate presence of *Helicobacter pylori* specific DNA in pancreatic adenocarcinoma using the PCR technique.

Material and method: The archive of the Pathology Department was screened to identify patients who had undergone Whipple surgery or distal pancreatectomy for initial diagnosis of pancreas carcinoma between June 2012 and June 2015. Formalin-fixed, paraffin embedded (FFPE) blocks of the patients were obtained from the pathology department. FFPE samples were taken from the pancreatic adenocarcinoma (PC) and peripheral pancreas tissues of PC. *H. pylori* DNA extraction was performed from the FFPE of these tissue samples using QIAamp DNA FFPE Tissue Kit (Qiagen, Germany) and presence of *H. pylori* was investigated using Genosen's *H. pylori* RG Kit.

Results: Forty-eight patients with PC (M/F, 28/16; mean, 48 years old) were included in the study. The histopathologic examination of surrounding the tumor; 19 patients had chronic pancreatitis, 15 patients had pancreatic intraepithelial neoplasia (PanIN), and 8 patients had normal pancreas tissue. *H. pylori* specific DNA was not detected in pancreatic adenocarcinoma and also surrounding tissue samples of 48 patients.

Conclusion: While there are several studies demonstrating relationship between *H. pylori* and pancreas cancer, we did not detect *H. pylori* specific DNA in pancreas adenocarcinoma tissue samples and peripheral tumor pancreas areas.

P-0272

Serum carbohydrate antigen 19-9 (CA 19-9) in the diagnosis of malignant obstructive jaundice

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Background: Carbohydrate antigen 19-9 (CA 19-9) is a tumour marker for malignancies of the hepatobiliary tract and pancreas, frequently elevated in a number of non-malignant conditions. This study was designed to assess the clinical application of CA19-9 in diagnosing pancreatobiliary malignancies in patients with obstructive jaundice and in discriminating between benign and malignant causes. **Materials and methods:** Sixty-three patients (30 benign and 33 malignant) presented with obstructive jaundice with elevated CA 19-9 were included in this study. Age 53.76 ± 14.48 years (mean ± SD) and sex 32:31 (M:F). Serum CA 19-9 levels were measured on admission and two weeks following biliary drainage by ERCP.

Results: The median value of CA 19-9 in malignant cases was higher (1000 U/ml) than benign cases (93 U/ml) (p = 0.001). After biliary drainage serum CA19-9 levels normalized in 15 (50 %) benign and only 1 (3 %) malignant cases (p = 0.001). Diagnostic accuracy of CA19-9 in the detection of malignancy was estimated by the receiver operating characteristic (ROC) curve. The AUC of CA 19-9 was 0.825. Sensitivity, specificity, PPV and NPV at cut off value 90 U/ml were 100, 50, 68.8 and 100; at 100 U/ml were 100, 53.3, 70.2 and 100; at 200 U/ml were 90.9, 66.7, 75 and 87, at 500 U/ml were 63.6, 76.7, 75 and 65.7 respectively.

Conclusion: This study showed that caution is necessary in the interpretation of an elevated serum CA 19-9 as a marker of pancreatico-biliary malignancy, especially in patients with benign cholestasis. Diagnostic accuracy of CA 19-9 was observed more at cut off value 200.

P-0273

A case report of carcinosarcoma of the lower bile duct with polypoid growth

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Background: Carcinosarcomas of the bile duct are very rare tumors consisting of both epithelial and mesenchymal elements. We report a case of carcinosarcoma of the lower bile duct and its clinical, radiological and pathological features.

Methods: A 82-year-old woman was admitted to a hospital with a complaint of jaundice and for evaluation of liver dysfunction. Computed tomography showed a dilatation of the common bile duct without stenosis and an enhanced mass inside. Endoscopic retrograde cholangiography revealed a 3 cm-diameter filling defect accompanied by an irregular obstruction in the lower bile duct. Intraductal ultrasonography revealed a floating mass with a stalk inside. Based on pathological examinations of the lower bile duct biopsy specimen, the tumor was diagnosed as a carcinoma. And pylorus-preserving pancreaticoduodenectomy was performed.

Results: Histological examination by light microscopy showed polypoid growth like 1p polyp with cartilaginous change and a transition between the carcinomatous and sarcomatous components with positive immunoreactivity for epithelial markers and mesenchymal markers respectively. It was diagnosed as carcinosarcoma of the lower bile duct. The patient is alive for 6 months without recurrence after the surgery.

Conclusions: The carcinosarcoma with polypoid growth of the bile duct is extremely rare. Our case suggests one of the findings of carcinosarcoma of the bile duct with presenting other reports.

P-0274

Percutaneous cholecystostomy as definite management for acute cholecystitis with klastin tumor

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Objectives: Ultrasound guided percutaneous cholecystostomy is an effective treatment for acute cholecystitis in critically ill patients. However, there has not been clearly established as definite management of cholecystitis instead of interval cholecystectomy especially in patient with unresectable klastkin tumor. In this study, we evaluated efficacy of percutaneous cholecystostomy as definite management for acute cholecystitis in patients with unoperable klastkin tumor.

Methods: From June 2009 to September 2015, we reviewed 49 consecutive patients with klastkin tumor, diagnosed as acute cholecystitis who received ultrasound guided percutaneous cholecystostomy, retrospectively. Long-term outcomes following percutaneous cholecystostomy were evaluated.

Results: 49 patients with a mean age of 74 years were identified. The technical and clinical success rates for percutaneous cholecystostomy were 100 %. All patient did not receive an interval cholecystectomy. The mean maintenance period of cholecystostomy tube was about 11.5 weeks (range 3–43). Two patients (4.1 %) suffered tube-related complications, including catheter displacement, bile leakage with site infection. 23 patients (46.9 %) could removed the cholecystostomy tubes during the follow up period. Seven out of 23 patients (30.4 %) suffered recurrent cholecystitis during the follow-up period. The mean time to re-intervention was 8.5 weeks (range 4–20). They were treated successfully with repeated percutaneous cholecystostomy.

Conclusion: Although some proportion of patients had recurrent attacks, percutaneous cholecystostomy as definite treatment for the management of acute cholecystitis seems to be one of feasible and considerable options in some patients with unoperable klastkin tumor.

P-0275

Liver function correction in pancreatic cancer patients with obstructive jaundice

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Purpose: To study the influence of conservative therapy in patients with pancreatic cancer complicated with obstructive jaundice.

Materials: A prospective analysis of liver function correction therapy in 45 patients with pancreatic cancer complicated with obstructive jaundice T3-4N0-1M0-1. Percutaneous decompression of the biliary tract was performed in 32 cases, retrograde stenting with plastic prosthesis in 13. Postoperative therapy included colloid plasma substitutes based on hydroxyethyl starch and protein emulsions with antibacterial and local hemostatic therapy background. In addition to improve the rheological properties of bile and liver function liver protectors and ursodeoxycholic acid medications were used.

Results: Relative hypovolemia and hypoproteinemia with hypercoagulation was observed on second postoperative day. Basically it is most clearly expressed in patients with baseline bilemia more 150 mmol/l. In addition, hepatocytes autolysis with more than 2.5 times intracellular enzyme rates increasing was observed. The average volume of allocated bile via external drainage was 317 ml/day. After therapy application there was a positive trend towards the normalization of enzymatic indicators. The average volume of allocated bile was increased up to 533 ml/day. In the study of bile biochemical composition and viscosity was found that the viscosity of the bile completely normalized on the tenth postoperative day, but with the preservation of the relative unbalance between free and bound bilirubin components, which indicates the presenting of latent hepatic insufficiency. Thus, our analysis shows the effectiveness of multicomponent conservative therapy in patients with pancreatic cancer after biliary tract decompression. Using of hepatotropic therapy improves the rheological properties of the bile and restores of hepatocyte functional activity.

P-0276

Pancreaticoduodenectomy in a huge pancreatic neuroendocrine carcinoma of infancy, case report

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Background: Pancreatic neuroendocrine carcinoma is extremely rare in pediatric population. Huge abdominal mass is one of the most common presenting symptoms of malignant tumour in infant. Surgical resection demonstrated the best survival for treatment of NETs.

Method: We present a nine month-old boy with huge right abdominal mass. Ultrasonography of the abdomen showed a large well-defined heterogeneous mass in the right abdomen measuring 9 cm in wide. MRI demonstrated abnormal large well-defined mixed solid-cystic lesion at right retroperitoneal region crossing midline of the abdomen, measured 8.9 × 8.8 × 9.7 cm in size, which was first diagnosed with pancreatoblastoma.

Results: We had performed pyloric preserving pancreaticoduodenectomy by using modified Blumgart's pancreaticojejunostomy anastomosis. A duct-to-mucosa was constructed under internal pancreatic duct stent without sutured by using a cut ETFE sheath of the SAFELET CATH 24G. The pathology revealed pancreatic neuroendocrine carcinoma with a Ki-67 index of 50 %. Immunostaining for chromogranin A and synaptophysin were positive, whereas CK7 and CK20 were negative. Post operative is uneventful. He received combined etoposide and cisplatin for adjuvant chemotherapy. After chemotherapy, MRI revealed no evidence of tumour recurrence.

Discussion: Pancreaticoduodenectomy is a feasible, and safe for the radical treatment of PNEC in infant. Modified Blumgart's pancreaticojejunostomy with duct-to-mucosa constructed under internal pancreatic duct stent without sutured is feasible for very small pancreatic duct.

P-0277

Changes of the humoral immune response in CHB patients with PEG-IFN therapy

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Hepatitis B antigen (HBeAg) seroconversion constitutes a significant milestone in the treatment of HBeAg-positive patients with chronic hepatitis B (CHB), but studies have yet to identify the specific humoral immune mechanisms behind the process or any accurate markers that can determine the virus-host immune status and, thereby, predict the degree of HBeAg seroconversion achievable. In the present longitudinal study, HBeAg-positive CHB patients were treated with PEG-IFN, higher frequencies of circulating CXCR5+CD4+ T cells and CD19+CD38+ plasma cells were found in patients in whom HBeAg seroconversion had been achieved. Both cell types peaked at 24 weeks for the HBeAg seroconversion group, while showing only a slight variation in the HBeAg non-seroconversion group. Furthermore, the frequency of PD-1+CXCR5+CD4+ T cells presented a general downward trend in the HBeAg seroconversion group, while this frequency was found to increase in the HBeAg non-seroconversion group. In addition, the frequency of PD-L1+CD19+CD38+ B cells demonstrated a larger decline in the HBeAg seroconversion group, comparatively. Furthermore, the ratio of circulating CXCR5+CD4+ T cells at 24 weeks and hepatitis B surface antigens (HBsAg) at 12 weeks was explored in terms of its ability to predict HBeAg seroconversion.

Conclusion: Dysfunction of the humoral immune response mediated by CXCR5+CD4+ T cells is associated with the failure of HBeAg seroconversion by way of PD-1/PD-L1. The CXCR5+CD4+ T cells/HBsAg ratio is an ideal marker for predicting HBeAg seroconversion in CHB patients.

P-0278

Successful immunological responses against hepatitis B virus in immunologically humanized mice

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Background and aims: Immunologically humanized mouse is required for the immunological study of hepatitis B virus (HBV). Recently, we generated *NOG-Iaβ/β2 m* double KO mice which were NOG mice deficient in both MHC class I and II (DKO-NOG mice). In this study, we established a new PBMC-engrafted humanized mouse model to demonstrate successful induction of human immune responses to HBV in mice.

Methods and results: We used NOG (*NODShi.Cg-Prkdc^{scid} Il12rg^{tm1sug}*) mice and DKO-NOG mice. After injection of human PBMCs, both hepatocyte damage and serum ALT elevation were observed in NOG mice, but not in DKO-NOG mice. Seven of eight NOG mice died within 2 months after injection of human PBMC whereas all DKO-NOG mice survived more than 70 days. On day 28 after injection of human PBMC, replacement rates of human immune cells in the liver increased up to 85 % in DKO-NOG mice. On day 15, the expressions of PD-1 and Tim-3 on T cells from DKO-NOG mice were significantly lower than those from NOG mice. Next, we evaluated the induction of HBV-specific cytotoxic T lymphocytes (CTLs) in humanized DKO-NOG mice. Both vaccination of Hbc-derived peptide-pulsed DCs and hydrodynamic injection of HBV DNA resulted in significant increase of Hbc-derived peptide-specific CTLs. Finally, we evaluated the production of anti-HBs antibody (anti-HBs) in sera of human PBMC-engrafted DKO-NOG mice. Inoculations of recombinant hepatitis B vaccine resulted in the production of anti-HBs in 2 of four vaccinated mice.

Conclusion: The present study demonstrates that immunological responses against HBV could be induced in the human PBMC-engrafted DKO-NOG mice.

P-0279

Analysis Th17/Treg balance and specific transcription factor(RORγt,Foxp3) in CHB patients

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Background: A large number of studies have shown that the pathogenesis of hepatitis B is the result of interaction of cellular immunologic response and virus 2.

Methods: 41 cases were mild chronic hepatitis B patients, 20 cases were moderate to severe hepatitis B patients. Select 20 cases who were healthy as control group at the same time. The frequency of Th17 cells and Treg cells in the peripheral blood of the patients group and control group were detected by flow cytometry. Peripheral blood transcription factor (retinoid-related orphan nuclear receptor γ t, RORγt) and (forkhead family transcription factor 3, Foxp3) were detected by real time quantitative *RT-PCR*. Statistic analysis of all data was conducted by the SPSS17.0 statistical software.

Results: (1) ROR γ t mRNA level and Foxp3mRNA level of CHB patients peripheral blood were higher than the control group; ROR γ t mRNA level and Foxp3mRNA level of CHB patients peripheral blood of moderate or severe group was higher than the mild group and control group, but when the mild group compared with the control group, RORγt mRNA level and Foxp3mRNA level of CHB patients peripheral blood had not obvious difference. (2) Th17/Treg cells ratio in CHB patients was higher than the control group, Th17/Treg ratio of the moderate to severe group was higher than the mild group.

Conclusion: Th17/Treg balance is broken in CHB patients peripheral blood. So that level of specific transcription factor (ROR γ t, Foxp3) of them change. That may become one of the causes of HBV chronicity and persistent infection.

P-0280

Analysis Th17/Treg balance and correlation with liver function, HBVDNA in CHB patients

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Background: By analysis correlation Th17/Treg imbalances with liver function and HBVDNA, providing new idea for treatment CHB patients, further to prevent cirrhosis and liver cancer associated hepatitis B.

Methods: 41 cases were mild chronic hepatitis B patients, 20 cases were moderate to severe hepatitis B patients. Select 20 cases who were healthy as control group at the same time. The frequency of Th17 cells and Treg cells in the peripheral blood of the patients group and control group were detected by flow cytometry. HBV markers were detected by the ELISA way. The liver function indicators such as ALT, AST, TBIL, were analyzed by the automatic biochemical analyzer. The HBVDNA levels of the patients group were tested by real-time fluorescent quantitative PCR analyzer.

Results: Th17/Treg cells ratio in CHB patients is higher than the control group, Th17/Treg ratio of the moderate to severe group was higher than the mild group; Th17/CD4+ T cells ratio of the mild and the moderate to severe group were positively related to the level of ALT. Th17/CD4+ T cells ratio was positively related to Treg/CD4+ T cells ratio in CHB patients; Th17/Treg cells ratio of HBeAg(+) group was positively correlated with ALT, AST levels. Th17/Treg cells ratio of HBeAg(-) group were positively correlated with TBIL level.

Conclusion: Treg cells increase was less than Th17 cells increase, resulting to Th17/Treg balance is broken, immune pattern of Th17/Treg moved to Th17 cells. Increased Th17 cells may be one of main reasons of causing the liver inflammation, liver cell damaging, and is closely related to severity of CHB.

P-0281

CTL mediated acute liver failure and rescue by CTLA4Ig in human hepatocyte transplanted TKNOG mice

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Background: HBV infection occasionally causes massive liver damage. We established a hepatitis B animal model using human hepatocyte transplanted TK-NOG mice and human PBMCs, and analyzed the effect of CTLA4Ig on liver damage.

Methods: After establishment of HBV infection, mice were inoculated with PBMCs isolated from a patient who recovered from acute

severe hepatitis B. Human serum albumin (HSA), HBV DNA, ALT, and cytokine levels were analyzed. Additionally, histopathological and flow-cytometric analysis of infiltrating cells were performed. HBV infected mice received intraperitoneally 1.5 mg of CTLA4Ig before and after PBMC transplantation.

Results: In HBV-infected and PBMC-transplanted mice, massive hepatocyte damage and decline in HSA and HBV DNA levels were seen. The population of regulatory T cells reduced and HBV-specific CTLs were detected by tetramer. Serum ALT, granzyme A and interferon-gamma levels were elevated. Two weeks after PBMC transplantation, the levels of HBsAg decreased below the detectable limit, and HBsAb became positive in all mice. CTLA4Ig treatment dramatically inhibited the decline of both HSA and HBV DNA levels compared to control, and histological examination also revealed neither invasion of mononuclear cells nor liver cell damage. Reflecting the inhibition of hepatitis, increase of serum ALT, granzyme A, and interferon-gamma levels was not seen after CTLA4Ig treatment.

Conclusions: We established an animal model of fulminant hepatitis caused by HBV infection using human hepatocyte transplanted TK-NOG mice inoculated with human PBMCs. Moreover, CTLA4Ig treatment strongly suppressed hepatitis. This animal model is useful for virological and immunological analysis of HBV infection.

P-0282

The research of Th17/Treg in patient with HBV-infected and its relationship with liver function

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In this research, we set the healthy adults (HC) as the control group, select the HBV-related liver diseases patients as experimental group, including asymptomatic carriers (AsC), chronic hepatitis B (CHB), liver cirrhosis (LC), hepatocellular carcinoma (HCC), investigate the Th17/Treg variations in the peripheral blood and its relationship with liver function. Th17/Treg in group of LC is higher than the group of HC, AsC, CHB and HCC, at the same time, the group of CHB is higher than the group of HC, AsC and HCC, which show the inflammation make progress from CHB to LC. The peripheral blood of Treg, Th17/Treg in group of HCC is higher than the group of HC, AsC, CHB and HCC, indicating that Treg has closely relationship with the occurrence and progress of HCC.

P-0283

Geranylgeranylacetone exerts anti-hepatitis B virus activity by suppressing enhancer-1 activity

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Aim: Geranylgeranylacetone (GGA) is isoprenoid compound, which has been used as an anti-ulcer drug. We previously reported that GGA has anti-hepatitis C virus activity in human hepatoma cells. However,

anti-hepatitis B virus (HBV) activity by GGA has not been studied. Aim of this study was to investigate the anti-HBV activity of GGA. **Materials and methods:** Linear HBV from patient sera was introduced into human hepatoma Huh7 cells according to the method described by Ganther et al. Transfected Huh7 cells were cultured in the presence or absence of GGA (100 μ M) for 72 h. The levels of HBs, HBe, HBc, mRNA and covalently closed circular (ccc) DNA were measured by real-time PCR. We made a luciferase vector inserted the amplified HBV enhancer-1/X promoter region. It was introduced to Huh7 cells and HBV enhancer-1 activities were measured by luciferase assay. Next, HNF3 α mRNA expression was assessed. Finally, HBV transfected human hepatocytes isolated from chimeric mice (PXB-cells) were treated with GGA (100 μ M). HBs and HBe Ag levels in supernatant were measured by chemiluminescent immunoassay (CLIA).

Result: GGA decreased HBs, HBc, and HBx mRNA levels in the Huh7 cells to 48, 39, and 50 %, respectively. However, GGA treatment did not alter ccc DNA levels. GGA decreased HBV enhancer-1 activity in Huh7 cells to 54 % and HNF3 α mRNA levels was reduced to 44 % compared to untreated cells. GGA decreased HBs and HBe levels in the PXB-cells to about 80 % respectively.

Conclusion: GGA reduces HBV related protein and mRNA by suppressing HBV enhancer-1 activity.

P-0284

Effect of cytokine IL-18 on different phases of chronic hepatitis B infection. progression

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Immune-mediated mechanisms have been found to play an important role in the progression of hepatitis B virus (HBV) infection. The outcomes of infection do not appear to be determined by viral strains. Instead, allelic variants in human genome are likely to affect the disease progression. In this study we have tried to investigate the role of the cytokines IL-18 in the hepatitis disease progression. The polymorphism of cytokines has been found to play important controlling roles in determining HBV infection outcome. The polymorphism study was done using allele specific PCR for the selected genes. A total of 210 samples were taken including all the four groups of HBV infection. PCR was done to obtain the desired result which was then subjected to gel electrophoresis. It was observed that the genotype frequency and the allelic frequency for all the sites examined were significantly distributed. In case of IL-18-137

CG genotype was dominant in case of IT (immunotolerant) group and GG genotype in all the other groups. For IL-18-607 CC genotype was present dominantly in all the groups except inactive carrier whereas AA genotype favoured inactive carrier phase. In case of IL-18-607 A allele was found to have a protective effect on the disease progression. It can thus be concluded that the genetic constitution of the host plays important role in the development of disease. However to completely establish the relation more extensive studies need to be done.

P-0285

A large scale screening of HLA-binding peptides from HBs and HBc peptide libraries

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Objectives: Polymorphisms in the human leukocyte antigens (*HLA*)-*DP* are one of the major susceptibility loci with chronic hepatitis B virus (HBV) infection [Kamatani et al. *Nat Genet* (2009); Nishida et al. *PLoS One* (2012)]. *HLA-DPB1*05:01* and **09:01*, the major *HLA-DP* alleles in East Asian populations, are associated with susceptibility, while *HLA-DPB1*04:01* and **04:02*, which are found at high frequencies in European descendants, confer protection against chronic hepatitis B. To elucidate the mechanisms of *HLA* association with chronic hepatitis B, we screened *HLA-DP*-binding peptides from the HBV surface antigen (HBs) and core antigen (HBc).

Methods: *HLA-DP* haplotypes that are associated with susceptibility to or protection against chronic hepatitis B were used in this study. Peptide libraries were designed for the entire sequences of the HBs and HBc, based on the consensus sequence of the genotype C. The *HLA-DP* binding peptides were screened through the in vitro peptide binding assay that used recombinant *HLA-DP* protein and synthetic peptides, as well as the cell-surface *HLA* expression assay, in which *HLA-DP* were expressed in fusion with the peptides.

Result: Through the in vitro *HLA*-peptide binding assay, we identified several regions that bound strongly to certain *HLA-DP* allele products. In addition to these regions, multiple other sequences that potentially bind to *HLA-DP* have also been identified through the cell-surface *HLA* expression assay. Based on these findings, we discuss potential roles that *HLA-DP* might play in the immune responses against HBV.

P-0286

Regulatory B cells increase in immune reactive phase of chronic hepatitis B virus infectionGuiyang Wang¹, Yong Liu², Zhenhua Sun², Ran Su¹, Rui Huang¹, Juan Xia¹, Yali Xiong¹, Xiaomin Yan¹, Zhaoping Zhang¹, Chao Wu¹¹Department of Infectious Diseases, Nanjing Drum Tower Hospital, Nanjing University Medical School, Nanjing, Jiangsu, China;²Department of Laboratory Medicine, Nanjing Drum Tower Hospital, Nanjing University Medical School, Nanjing, Jiangsu, China**Background:** Regulatory B cells (Bregs) are reported to play an important role in the immune responses to chronic HCV infection via toll-like-receptor (TLR) signaling. We aimed to investigate the characteristics of Bregs and expression of TLRs on Bregs in the peripheral blood of patients with chronic HBV infection.**Methods:** One hundred and thirty-one patients with chronic HBV infection and 22 healthy controls (HC) were enrolled. The patients were divided into three groups, namely immune tolerant phase (IT), immune reactive phase (IA) and inactive HBV carrier state (IC) based on HBeAg status, HBV DNA and ALT levels. The frequencies of Bregs (defined as CD24^{hi}CD38^{hi} B cells) and expression of TLR2, TLR4 and TLR9 on Bregs in the peripheral blood were measured by flow cytometry.**Results:** Compared to HC group (3.811 ± 1.906 %), the frequencies of Bregs were significantly elevated in the CHB group (6.343 ± 32.287 %, P < 0.001). Furthermore, the frequencies of Bregs were different in various immune phases of chronic HBV infection. The frequencies of Bregs were significantly elevated in the IA group (7.565 ± 3.607 %, P < 0.001) compared to IT group (4.643 ± 2.633 %, P < 0.001) and HC group (3.811 ± 1.906 %, P < 0.001). However, there were no significant differences between the expression of TLR2, TLR4 and TLR9 in various immune status of chronic HBV infection.**Conclusions:** Bregs may play some important role in modulating the immune responses of chronic HBV infection. However, whether Bregs modulate immune responses via TLR signaling in chronic HBV infection remains ambiguous.

P-0287

Polyclonal B cell activation induced by Tfh correlated with Th17 mediated liver damage in CHB

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Background: B-cell polyclonal activation is involved in pathogenesis during chronic virus, such as HIV and HCV infection; however, its role in chronic hepatitis B (HBV) infection remains unknown.**Methods:** Here we characterized peripheral and intrahepatic B cells and revealed the cause and clinical significance of B-cell polyclonal activation during chronic HBV infection. Patients with chronic HBV infection in different phases: immune tolerance (IT, n = 12), immune activation (IA, n = 43), and healthy controls (HC, n = 22) were enrolled.**Results:** The IA patients displayed an overall polyclonal activation indicated by higher percentages of peripheral blood B cells, higher levels of activation markers and secretion of IgG and IgM. In addition, serum IL-21 and circulating CXCR5 + CD4 + Tfh cells werealso increased in IA patients, which were positively correlated with polyclonal activation of B cells. Tfh cells, in vitro, can induce B-cell polyclonal activation through IL-21 production. Furthermore, B cells can facilitate production of IL-17 + CD4 + T cells (Th17) which was depended on secretion of IL-1 β and IL-6. In addition, B cells and interleukin-17 (IL-17) producing cells were concurrently increased in situ liver in IA patients and B cells were showed positive correlation with IL-17 producing cells and histological activity index.**Conclusion:** B cells are polyclonal activated in peripheral blood and accumulated in livers of IA patients, and exhibit a potential to exacerbate liver damage during chronic HBV infection.

P-0288

Analyzing RNA exosome mediated HBV-RNA degradation mechanism

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Interferon system is required for efficient induction of both innate and adaptive immune response. As a mechanism to evade the anti-viral host immune response, hepatitis B virus (HBV), is known to minimally induce the interferon system. However, 90 % of acute infected adults can still clear the virus; suggesting the possible presence of other interferon-independent pathways leading to viral clearance. We have identified SKIV2L/RNA exosome system to efficiently degrades HBV-RNA and suppress HBV life cycle, even in the absence of the interferon induction. Further analysis of this mechanism is required to develop novel anti-HBV approach that can target this pathway and suppress HBV life cycle. In this meeting, we will report our data regarding the mechanistic analysis of this pathway.

P-0289

Role of TLR8 mediated antiviral immunity in chronic hepatitis B

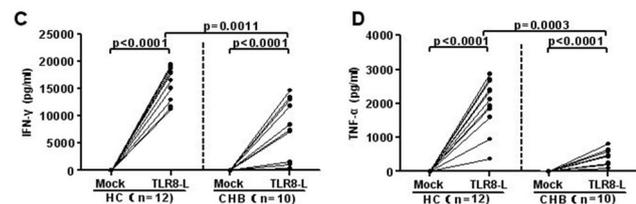
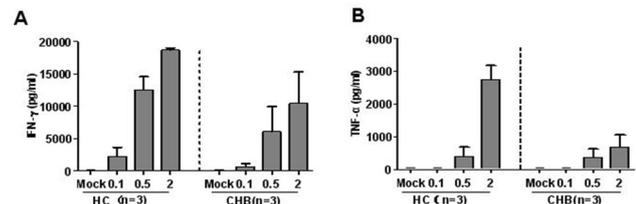
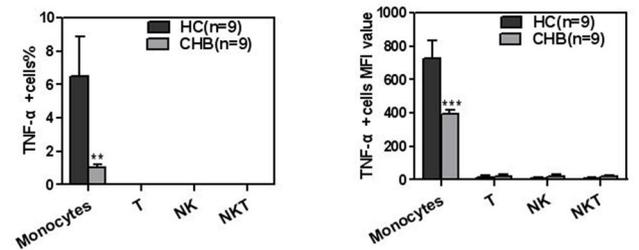
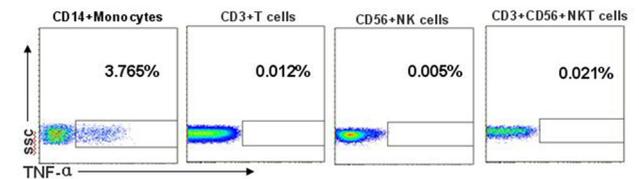
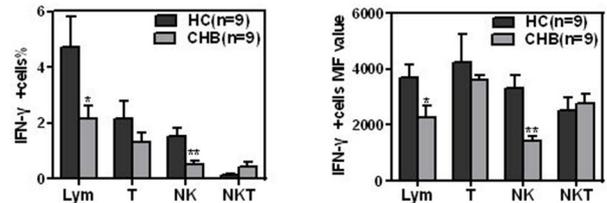
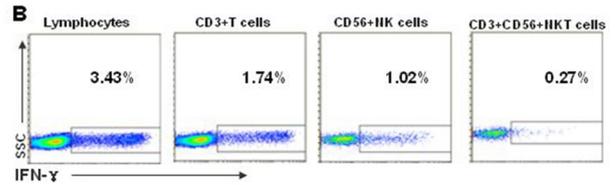
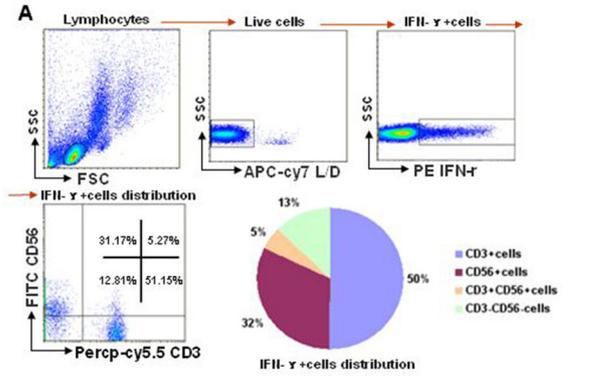
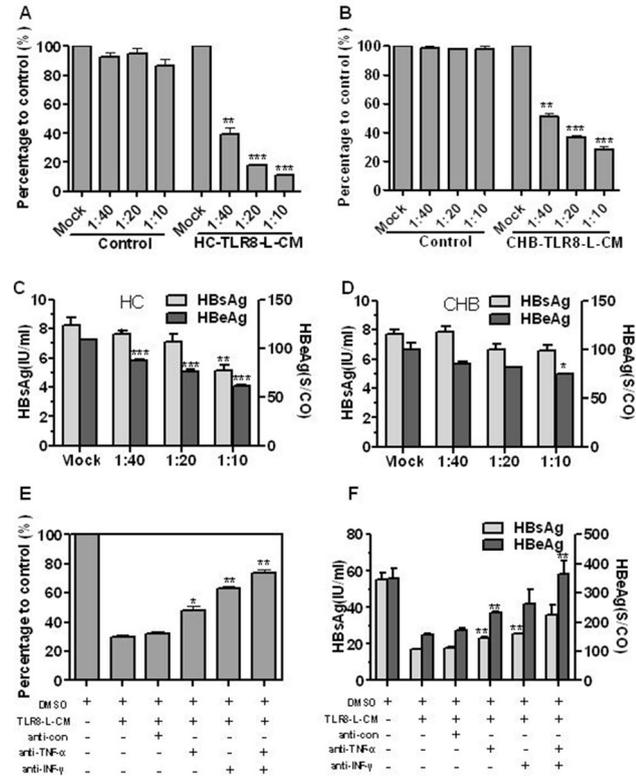
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Background: The activation of TLR8 signal pathway induces intracellular signaling pathways leading to the production of inflammatory cytokines facilitating the defense of various invading pathogens. Until recently, the contribution of TLR8 to innate immunity remained poorly understood under the condition of HBV infection, as this TLR was initially considered to be dysfunction in murine. Here we investigated the molecular mechanism that contributes to the antiviral response to TLR8 ligand in vitro.**Methods:** Antiviral activity of TLR8-ligand ssRNA40 to HepG2.2.15 was analyzed by real-time PCR, ELISA and antibodies neutralization assay.**Results:** The results showed that exposure of HepG2.2.15 to the conditioned media (CM) from HC and CHB PBMCs stimulated by ssRNA40 strongly reduced the levels of HBV DNA in a dose-dependent manner, while addition of IFN- γ or TNF- α neutralizing antibody into CM blocked the antiviral effect. Interesting, more pronounced inhibition of HBV replication was observed in CM from HC, as compared to CHB. A survey of cytokines showed that

ssRNA40 induced a massive production of IFN- γ and TNF- α which were degraded under the infection of HBV compared with HC ($p < 0.01$). Further, intracellular cytokine staining of PBMCs cultures revealed that NK cells and T cells served as the principal IFN- γ -producing lymphocytes, while monocytes were the main source of TNF- α .

Conclusions: In conclusion, TLR8 exerts its inhibitory effect on HBV replication by IFN- γ and TNF- α secretion, though is weaker in CHB patients, which may open new therapeutic opportunities for the treatment of chronic HBV infection.



P-0290

HBXIP negatively regulates antiviral response by promoting proteasomal degradation of TBK1

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TANK-binding kinase 1 (TBK1) plays an essential role in Toll-like receptor (TLR) and retinoic acid inducible gene I (RIG-I) mediated induction of type I interferon (IFN; IFN- α/β) and host antiviral responses. How TBK1 activity is negatively regulated remains largely unknown. We report that hepatitis B virus X-interacting protein (HBXIP) promotes proteasomal degradation of TBK1 and inhibits RIG-I-induced IFN- β signaling. HBXIP knockdown resulted in augmented activation of IFN regulatory factor 3 (IRF3) and enhanced expression of IFN- β in RIG-I-activated primary hepatocyte cells, whereas overexpression of HBXIP had opposite effects. Consistently, HBXIP impaired vesicular stomatitis virus (VSV) infection induced IRF3 activation and IFN- β production and promoted VSV replication. HBXIP negatively regulated the cellular levels of TBK1 by directly binding to and promoting K48-linked polyubiquitination of TBK1. Therefore, we identified HBXIP as a negative regulator in RIG-I triggered antiviral responses and suggested HBXIP as a potential target for the intervention of diseases with uncontrolled IFN- β production.

P-0291

Mechanisms of interaction between host glycosylation system and hepatitis B virus replication system

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Background/Aim: It is important to investigate the mechanism between host glycosylation system and hepatitis B virus (HBV) replication system to understand the pathology of chronic hepatitis B viral infection. The aim of this study was to investigate the mechanisms of interaction between host glycosylation system and HBV replication system to find candidate molecules for developing new anti-viral agent against HBV.

Methods: HepG2.2.15 cells which are consistently producing HBV were employed for our in vitro study. We have investigated the effects of various siRNAs targeting glyco-genes on HBV replication using siRNA screening panel. The effect of single sequence of siRNA was investigated, when the siRNA mixture targeting some glyco-gene was effectively impaired HBsAg and HBV DNA secretion at the first screening. Results: Some siRNA targeting the glyco-genes inhibited the secretion of HBV in the culture supernatant. Especially, one specific siRNA targeting the glyco-gene which is responsible for one of the nucleotide sugar transporter (NST) has strongly reduced the titer of HBV DNA and almost completely blocked HBsAg secretion in the culture supernatant. Moreover, the siRNA showed that the reduction of the accumulation of HBsAg in the cells by immunoblotting.

Conclusions: Some siRNA targeting glyco-genes, especially inhibition of the certain NST gene, strongly inhibited the HBsAg secretion. Knocking down one of the NST gene did not show the accumulation of the HBsAg, therefore, it may inhibit the production of the envelope

proteins. Carbohydrate synthesis system associated with the life cycle of HBV could be a potent target for developing novel anti-HBV agents.

P-0292

SKIV2L/RNA exosome mediated regulation of hepatitis B virus

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Hepatitis B virus (HBV) is a stealth virus, minimally inducing the Interferon system required for efficient induction of both innate and adaptive immune response. However, 90 % of acute infected adults can clear the virus; suggesting the possible presence of other interferon-independent pathways leading to viral clearance. Because of its ability to bind and identify viral nucleic acids, many helicases were found to play an important role inducing the anti-viral state. Using arrayed shRNA targeting 133 human helicases, we performed a functional screening to identify those who suppress HBV replication. We here report superkiller viralicidal activity 2-like (*S. cerevisiae*) (SKIV2L) RNA helicase dependent mechanism that regulates HBV replication through preferential degradation of HBV-RNA. This mechanism is interferon-independent. RNA immunoprecipitation analysis identified the formation of SKIV2L/HBV-RNA complex, which is regulated by SKIV2L's phosphorylation at serine residues 243 and 245 amino acids, and the further association of this complex with the RNA exosome proteins. The binding between SKIV2L and HBV-RNA was RNA exosome-independent, suggesting that SKIV2L first identifies HBV-RNA then shuttles it to the RNA exosome where it is degraded. SKIV2L also suppresses the Duck HBV (DHBV) but not HCV, suggesting that SKIV2L is a possible common effector molecule against different members of the Hepadnaviridae.

P-0293

HBV blocks MG132-induced apoptosis in human hepatic stellate cells

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Induction of apoptosis of human hepatic stellate cells (HSCs), which plays a role in hepatic fibrosis, might be one of the therapeutic strategies against HBV-related hepatic fibrosis. In the present study, the effects of HBV on apoptosis and endoplasmic reticulum (ER) stress signaling in HSCs were examined. We collected conditioned media (CM) from HepG2.2.15 and HepG2, and those from primary human hepatocytes infection with HBV. We cultured human HSC LX-2 with these CM or co-cultured LX-2 with HepG2 or HepG2.2.15. After 24 h, activation of AP-1 or JNK/c-Jun expression was evaluated by luciferase assay or western blotting, respectively. We examined the effects of HBV on MG132-induced apoptosis in LX-2 and HHStcC by APOPercentage assay and immunofluorescence study. We also examined the effect of over-expression or knock-down of c-Jun on MG132-induced HSC-apoptosis. The effects of HBV on 84 ER-stress-related genes were also examined by real-time PCR-based

array. (1) In LX-2, activation of AP-1 was inhibited by the addition of HBV-including CM. (2) This phenomenon was also confirmed by co-culture with HepG2.2.15. (3) Phosphorylated-c-Jun and c-Jun expressions were reduced in LX-2 with HBV. (4) MG132-induced apoptosis and both Annexin-V and γ -H2AX expressions were inhibited in LX2 with HBV. (5) Expression of cAMP responsive element binding protein 3-like 3, inhibin-beta A and solute carrier family 17-member 2 mRNAs was up-regulated in LX-2 cells with 2.2.15-CM. These could contribute to the inhibition of apoptosis of HSCs and cause hepatic fibrosis. HBV also upregulated several ER stress genes associated with cell growth and fibrosis.

P-0294

Aberrant methylation of inflammatory pathways in HBV infection

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Aims: So far, little is known about blood methylation profile during HBV infection. This study aimed to identify differentially methylated genes (DMGs) between diverse stages of HBV infection.

Methods: Three groups of subjects were recruited, including seven individuals with self limiting acute hepatitis (AH), 42 with chronic HBV infection (CH) and seven healthy controls (N). Whole genomic DNA and total RNA were extracted from peripheral blood mononuclear cells (PBMCs). DNA methylation array and whole-genome gene expression array were performed on Roche NimbleGen human DNA methylation 3 × 720k CpG Island plus refseq promoter array and Illumina human WG-v3 respectively.

Results: Mainly, DMGs between two comparing sets (AH vs N and AH vs CH) annotated in inflammation pathway are similar, which were TLR9, TNFRSF1A, CCL22, IL6, C1QB, CTLA4, LILRA1, LILRB1, LMO2, NCF1 and NCF4, however, some of which showed significantly hypomethylated levels in acute infection. Several tumor suppressor genes, like RASSF1, APC, CDKN2A, INK4B, MMP1, IRAK3 and CCND2 show hypermethylation status in chronic phase compared to healthy controls. When methylation data were reverse cross-linked with expression data, several key genes annotated in the T cell receptor and inflammatory process, like NFKB2, CTLA4, CD3E, MAPK3, TLR9 and IL6 significantly hypomethylated and hyperexpressed levels in acute phase comparing with healthy controls and chronic phase respectively.

Conclusions: Our study provided a comprehensive blood DNA methylation profile during HBV infection. The process of T cell receptor activation and TLR9 mediated inflammatory response may be regulated by aberrant DNA methylation.

P-0295

The characterization of HBV ccc DNA in placenta tissues from pregnant women with positive HBsAg

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Background/aim: HBV infection can cause multiple organ damage had been reported. However, it remains unclear whether HBV cccDNA replicates in extrahepatic tissues particularly in placenta tissues. The study is to explore the characterization of HBV cccDNA in placenta tissues.

Methods: The placenta tissues were obtained from 52 patients with chronic hepatitis B. The samples were fixed by 10 % formaldehyde and paraffin imbedded and treated with 0.05 % poly-L-lysine. The tissue sections were firstly treated by plasmid safe ATP dependent DNase (PSAD) so as to digest relaxed circular DNA (rcDNA) prior to RCA. Four pairs of primers were designed for mediating RCA for the first round amplification of HBV cccDNA. HBV cccDNA was further amplified by a pair of selective primers and digoxigenin labeled probes that targets the gap region between the two direct repeat regions (DR1 and DR2) of the virus after RCA. HBV DNA and HBV surface antigen (HBsAg) and HBV core antigen (HBcAg) were routinely performed.

Results: Of the 52 pregnant women with positive hepatitis B surface antigen and HBV DNA, HBsAg and HBcAg were not detected in the placenta tissues, moreover, none of the detected HBV cccDNA in the placenta tissues.

Conclusions: Placenta tissues may not support viral replication. Further large sample studies are needed to verify our findings.

P-0296

Inhibition of HBV cccDNA transcription by CPP-PNA conjugates assembled on oligonucleotide scaffolds

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HBV cccDNA persists in the host cell nucleus and drives a viral rebound once therapy is discontinued. Peptide nucleic acids (PNAs) hybridize to complementary sequences by Watson–Crick base pairing and have an outstanding ability to invade double stranded DNA. However, delivery to intracellular target sites is still one of the main obstacles in the development of PNAs as antisense-antigen therapeutics. In current study, we designed a self-assembled oligonucleotide scaffold that included a central complementary region for self-assembly and lateral regions complementing the PNAs. CPP-PNAs were assembled with the oligonucleotides scaffolds and tested for their activity targeting at different forms of HBV DNA, including cccDNA in HepDES19 cells, the pUC18-HBV1.2 plasmid transfected into HepG2 cells, and integrated-HBV DNA in chromosomal of HepG2.2.15 cells. Our results showed that assembly of cell-penetrating peptide (CPP)-PNAs on the scaffold significantly promoted endocytosis of PNAs by at least 10-fold in cell cultures, particularly for scaffolds in which the central complementary region was assembled by poly(guanine) and poly(cytosine). The antisense activity of CPP-PNAs increased by assembly on the scaffold and was further enhanced after co-assembly with endosomolytic peptide (EP)-PNA. This synergistic effect was also observed following the assembly of antigenic CPP-PNAs/EP-PNAs on the scaffold. However, antigenic activity was only observed by targeting episomal viral cccDNA or transfected plasmids, but not the chromosome in the cell cultures.

Conclusion: Oligonucleotide scaffolds provides a simple strategy for assembly of multiple functional peptide-PNA conjugates, expanding the applications of PNAs and demonstrating the potential of PNAs as antiviral agents targeting at HBV cccDNA.

P-0297

Serum level of microRNA in the patients with ACLF related HBV infection**Ya Wen**

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Aim: To identify the significant miRNAs and the influence factors of miRNAs in the patients with acute on chronic liver failure related hepatitis B virus infection.**Method:** Sera collected from 41 CHB, 55 HBV-associated acute-on-chronic liver failure (ACLF) patients and 30 chronic asymptomatic carriers (ASC) were included in this study. In order to demonstrate whether miRNAs may have been correlated with the severity of HBV-related disease, we used microarray to investigate the miRNA expression profiles in serum from ASC, CHB, ACLF patients. Those miRNAs with altered levels were further measured by quantitative reverse transcription polymerase chain reaction (qRT-PCR). The SPSS version 18.0 (SPSS Inc., Chicago, IL, USA) was used for the statistical analyses.**Conclusion:** 1. MiRNA-146a-5p, 122-3p, 328-3p can be novel non-invasive biomarkers in HBV infection. The more severe inflammation is, the higher the expression level is. 2. The prognosis of ACLF are associated with INR, Na⁺, MELD, hepatic encephalopathy, gastrointestinal bleeding, lung infection, hepatorenal syndrome, 122-3P, 146a-5p, 328-3p. The predictive model of the prognosis of ACLF include four aspects: Na⁺, INR, gastrointestinal bleeding, 122-3p, better than the MELD score. The new model is $Y = 0.402 \times Na^+ - 1.72 \times INR - 4.963 \times \text{gastrointestinal bleeding} - 0.278 \times 122-3p + 50.449$. 3. Four miRNA in each other have no cooperation in ACLF.

P-0298

MicroRNA-155 in peripheral blood mononuclear cells of patients infected with hepatitis B virus**Sulin Yu**

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Background: Persistent hepatitis B virus (HBV) infection is sustained due to inadequate natural and acquired immune responses. Recent studies have suggested that microRNA (miR)-155 is involved in immune cell differentiation and maturation, and affects immune responses to viral infection. However, little is known of the interaction between HBV and miR-155 in host antiviral immunity. To study the role of miR-155 in the immune response to HBV infection, we evaluated miR-155 levels in peripheral blood mononuclear cells (PBMCs) of chronic hepatitis B patients relative to that of healthy

subjects, and investigated an association between miR-155 levels and HBV DNA or ALT.

Methods: Total RNA was extracted from peripheral venous blood samples of 90 treatment naive patients with chronic HBV infection and 20 healthy volunteers. The levels of miR-155 in the PBMCs were measured by quantitative real-time PCR.**Results:** MiR-155 levels of the HBV patients were significantly lower than that of the healthy controls ($p = 0.001$). HBV patients with elevated alanine aminotransferase (ALT) had higher levels of miR-155 than did patients with normal ALT ($p = 0.014$). No correlations were found between miR-155 and ALT or HBV DNA.**Conclusions:** MiR-155 appeared suppressed during HBV infection. The significantly lower miR-155 levels in ALT-elevated HBV patients suggest that miR-155 levels in PBMCs correlate with the immune state of patients with chronic HBV infection.

P-0299

Lower circulating microRNA-122 indicate severe hepatic fibrosis in chronic hepatitis B patients**Masato Nakamura, Tatsuo Kanda, Yuki Haga, Reina Sasaki, Shuang Wu, Shin Yasui, Makoto Arai, Shingo Nakamoto, Fumio Imazeki, Osamu Yokosuka**

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Background and Aims: MicroRNAs (miRs) are small RNA particles consisting of 19–22 nucleotides. Recently, many researchers focus on circulating miRs in serum as biomarkers. It has been reported that hepatic expression of miR-122 decreases in chronic hepatitis B (CHB), suggesting the association between miR-122 and liver diseases. We assessed serum miR-122 levels in CHB and chronic hepatitis C (CHC) patients and compared them with their liver biopsy findings to uncover the usefulness of serum miR-122 as a biomarker.**Patients and Methods:** Ninety-one CHB and 108 CHC patients with liver biopsy, and 23 healthy controls were enrolled. After the addition of *Caenorhabditis elegans* miR-39 (cel-39) as spike-in control, miRs were extracted from serum. Serum miR-122 levels were measured by TaqMan real-time PCR assay with ddCt methods. Association between serum miR-122 and liver biopsy findings were evaluated.**Results:** Serum miR-122 levels of CHB and CHC patients were higher than those of healthy controls. In CHB patients, miR-122 of patients with severe fibrosis (F3 and F4) were lower than those with mild or moderate fibrosis (F1 and F2) (6.40 vs 4.36; $p < 0.05$); and miR-122 of patients with severe inflammation activity (A3) tended to be lower than those with mild or moderate inflammation (A1 and A2) (6.32 vs 4.78; $p = 0.097$). However, in CHC patients, miR-122 did not differ among different staging of fibrosis or grading of inflammatory activity (F1 and F2/F3 and F4: 9.07/8.48, $p = 0.26$; A1 and A2/A3: 8.96/8.15, $p = 0.21$).**Conclusion:** Serum miR-122 levels could predict hepatic fibrosis and hepatic inflammation in CHB patients.

P-0300

Do unfavorable rtM204I/V and sW182stop/rtV191I mutations trend to co-mutate in genotype C HBV?

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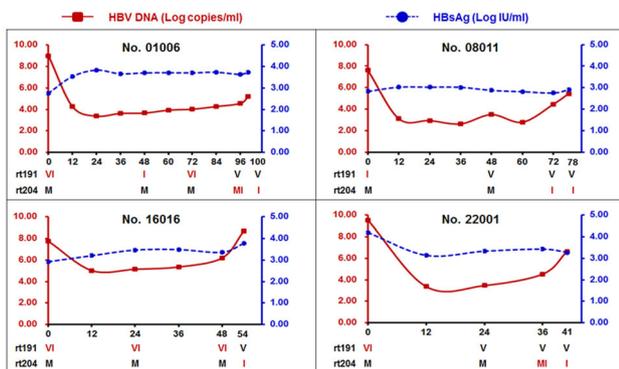
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Background: The rtM204I/V mutation in reverse transcriptase (RT) and sW182stop/rtV191I truncation mutation in hepatitis B surface antigen (HBsAg) are two important mutations conferring antiviral resistance and potential carcinogenesis to hepatitis B virus (HBV). The co-existence of them is still poorly studied.

Methods: Hepatitis B e antigen-positive chronic hepatitis B patients with genotype C infection were enrolled. The RT sequences overlapping sW182 were analyzed by different sequencing methods. The replication-competent plasmids containing rtM204I, or sW182stop/rtV191I single and combined mutations were constructed.

Results: In a cross sectional cohort, the detection rate of sW182stop/rtV191I was 13.95 % (6/43) in those with wild-type rtM204 and 0.00 % (0/58) in those with rtM204I/V mutations ($P = 0.005$). In four follow-up cases the vanishing of sW182stop/rtV191I was found to be accompanied by emergence of rtM204I/V along with lamivudine treatment by direct sequencing (Figure). Clone sequencing for two of them revealed that rtM204I/V + sW182stop/rtV191I mutation was not found within a sequence in 139 clones but rtM204I/V or sW182stop/rtV191I single mutation harboring clones could be found in one sample, which was then subjected for Illumina Miseq-based sequencing. The results showed that detection rates of rtM204I/V, rtV191I and rtM204I/V + rtV191I were 6.36 % (5293/83,242), 51.17 % (42,594/83,242) and 1.07 % (888/83,242). The HBsAg level of rtM204I + rtV191I plasmid in Huh 7 culture supernatant was below the lowest limit of detection (0.05 IU/ml), while those of rtM204I and sW182stop/rtV191I were detectable.

Conclusions: The finding that rtM204I/V + sW182stop/rtV191I mutations rarely exist in one HBV genome allays the worry of synergistic unfavorable effects of these two mutations.



P-0301

Analysis of human microRNAs profiles in response to HBV transfection and Peg-IFN treatment by NGSThananya Jinato¹, Pisit Tangkijvanich^{2,3}, Natthaya Chuaypen^{2,3}, Witthaya Poomipak¹, Kesmanee Praianantathavorn², Sunchai Payungporn^{2,3}

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Objectives: Interferons (IFNs) play important roles in defense mechanisms against viral infections, thus IFNs therapy is a standard treatment in chronic hepatitis B (CHB) patients. IFNs signaling pathways promote IFN-inducible genes including microRNAs (miRNAs). In this research, we aimed to determine microRNAs expression profiles in hepatocellular carcinoma cells line (Huh7) transfected with hepatitis B virus (HBV) plasmid and treated with pegylated interferon (Peg-IFNs) alpha 2a.

Materials and Methods: Huh7 cells (7×10^5 cells/dish) were transfected with or without a plasmid containing HBV genome (3 μ g) for 6 h followed by treatment with or without Peg-IFNs alpha-2a (100 ng/ml) for 24 h. Cellular small RNAs were extracted by microRNA purification kit (Norgen) and then followed by library preparation based on NEBNext[®] Small RNA Library Prep (NEB). To determine miRNAs expression profiles, the next-generation sequencing (NGS) was carried out on MiSeq platform (Illumina). The CLC genomic workbench software was applied for data analysis.

Results: Huh7 cells transfected with HBV plasmid and treated with Peg-IFNs alpha-2a were significantly down-regulated miRNAs including miR-128, miR-151, miR-185, miR-186, miR-425, and miR-483. The target genes of the candidate miRNAs were predicted in terms of roles in cellular pathways and immune response, which might be related to treatment in CHB patients.

Conclusion: This study revealed better insight into the effect of Peg-IFNs alpha-2a on alteration of miRNAs expression level in Huh7 cells. However, putative mRNA targets of these miRNAs should be further confirmed for better understanding about interplays between interferon response and microRNA expression in anti-viral defense.

P-0302

Circulating mir-150 and mir-342 as potential biomarkers to predict HCC progression in HBV patients

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Background: Circulating miRNAs have evolved as promising biomarkers for different cancers. The significance of circulating miRNAs in HBV related HCC remains poorly understood.

Aims: To explore novel miRNAs associated with HBV infection and their utility as non-invasive diagnostic biomarkers in HCC progression.

Patients and Methods: Forty eight patients; cirrhosis (n = 24), HCC (n = 24) and 29 healthy controls (HC) were enrolled. Plasma panel for 185 miRNAs was used to screen the differentially expressed miRNAs. Validation was carried out by rt-PCR.

Results: We identified 34 differentially expressed miRNAs in the patient groups compared to HC ($p < 0.05$) (fold change < 2). Of these, miR-150 and miR-342 presented significant downregulation in HCC ($p < 0.00001$) (fold change ≤ 25) and ($p < 0.001$) (fold change ≤ 15) respectively. Validation data implied, mir-150 and mir-342 were significantly downregulated in serum and matched tissues of HCC patients than cirrhosis ($p < 0.05$) and HC ($p < 0.001$). Further, ROC analysis revealed; serum miR-150 and miR-342 were potential markers for discriminating HCC patients from Cirrhosis and HC, with ROC curve areas of 0.86 (95 % CI 0.81–0.92), 0.89 (95 % CI

0.73–0.86) respectively in HC and 0.89 (95 % CI 0.78–0.95) and 0.90 (95 % CI 0.85–0.96 in cirrhosis, respectively). At the cut-off values >3.52 (for miR-342), 0.60 (for miR-150) the sensitivity and specificity for these markers were (84, 73.5 %) and (70.7, 69.1 %) respectively. Further, significant positive correlation between plasma and tissue miR-342 was found in cirrhosis $R^2 = 0.3$ ($p < 0.02$) and HCC $R^2 = 0.7$ ($p < 0.001$).

Conclusion: Our data signifies that serum miR-150 and miR-342 might serve as potential biomarkers for HCC detection.

P-0303

Pattern of hepato-pancreatic diseases in Cox's Bazar Medical College Hospital

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Aim: Hepatobiliary diseases result a significant burden to any country across the world. Pattern of Hepatobiliary diseases varies with geographical locations globally as well as regionally within a country. This study aimed to determine the pattern of liver diseases in a District Hospital in Bangladesh.

Methods: Hospital registry data of admitted male and female patients in the Medicine Ward over a period of 1 year from July 2013 to June 2014 were analyzed. All admitted patients with clear case identities and a recorded diagnosis were included in the study.

Results: Hepato-pancreatic diseases accounted for 3.9 % (male 61.1 % and female 38.9 %) of the admitted patients with recorded diagnosis. The most common types of patients were liver cirrhosis (33.42 %; male 43.0 %, female 18.2 %) and acute hepatitis (18.9 %; male 19.4 %, female 18.2 %). Biliary as cariasis also comprised a significant proportion (18.2 %; male 7.3 %, female 35.1 %).

Conclusion: Liver cirrhosis accounted for over one third of liver diseases in hospitalized patients in this hospital. Acute hepatitis and biliary ascariasis accounted for another one third of the patients in the specialty. A significant proportion of the diseases are preventable.

P-0304

The automated system for screening of liver disease

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Aim: the creation of an automated system for screening of liver disease.

Methods: The automated system (index test) allows to build a preliminary diagnosis by combination of values of laboratory tests (ALT, AST, AP, GGT, gamma-globulin, TB, anti-HCV, HBsAg), BMI, alcohol and medications intake. Reference test: final diagnosis followed by patient's examination. 474 completed case records were blinded and tested by index test.

Results: For all cases index test accuracy—97, 2 %; sensitivity—97, 2 %; specificity—31, 7 %; LR+: 68, 7 %; LR–: 83, 1 %. ALD index test accuracy—97, 1 %; sensitivity—86, 3 %; specificity—98, 2, 7 %; LR+: 82, 6 %; LR–L 98, 6 %. NAFLD index test accuracy—86, 2 %; sensitivity—67, 8 %; specificity—89, 7 %; LR+: 81, 2 %; LR–: 93, 5 %. Hepatitis C index test accuracy—56, 1 %; sensitivity—33, 2 %; specificity—98, 67 %; LR+: 97, 8 %; LR–: 44, 3 %. Hepatitis B index test accuracy—98, 7 %; sensitivity—100, 0 %; specificity—98, 6 %; LR+: 87, 5 %; LR–: 100, 0 %. Cholestatic liver disease index test accuracy—88, 7 %; sensitivity—65, 0 %; specificity—93, 0 %; LR+: 62, 7 %; LR–: 93, 6 %. DILI index test accuracy—99, 1 %; sensitivity—92, 8 %; specificity—99, 3 %; LR+: 81, 2 %; LR–: 93, 5 %. Autoimmune liver disease index test accuracy—79, 9 %; sensitivity—55, 5 %; specificity—82, 3 %; LR+: 23, 6 %; LR–: 94, 9 %. Other liver diseases index test accuracy—83, 5 %; sensitivity—20, 73 %; specificity—93, 9 %; LR+: 36, 1 %; LR–: 87, 7 %.

Conclusion: Index test for screening of liver disease creates the preliminary diagnosis with appropriate accuracy and can be useful to evaluate groups of risk for liver disease.

P-0305

A population-based study on the seroprevalence of viral hepatitis in Hong Kong, China

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Background: Viral hepatitis remains a major health burden in the Asia-Pacific region. Reliable virus prevalence data influencing countrywide health policies can only be obtained by population-based study which requires intensive efforts to conduct. We aimed to document the territorial seroprevalence of viral hepatitis A to E in Hong Kong.

Methods: Questionnaires containing demographics, family history of hepatitis infection, previous hepatitis vaccination and risks of hepatitis exposure are collected in different townships over Hong Kong. Sera are tested for antibodies to HAV, HCV and HEV, hepatitis B panel including HBsAg, HBeAg, anti-HBs and anti-HBc and anti-HDV for HBsAg positive subjects.

Results: We aim to recruit 10,000 subjects. Since study commencement in February 2015, a total of 4371 subjects (M:F, 32 vs. 68 %) were recruited. Mean age were comparable in both gender (male 53.0 ± 14.6 vs female 53.6 ± 14.1). Interim analysis of overall seroprevalence data of 4371 subjects were shown in figure 1. Significantly more patients are anti-HAV and anti-HEV positive in older age groups when compared to younger age groups ($p < 0.001$) as shown in Figure 1. Among 3983 HBsAg-negative subjects, 1593 subjects (59.9 %) were positive for anti-HBc, with statistically higher proportion of anti-HBc positivity in older age groups ($p < 0.001$) as shown in Figure 2.

Conclusions: Prevalence of HBV remained high. More than half of the HBsAg negative population had HBV exposure evidenced by anti-HBc positivity. Anti-HAV and anti-HEV were significantly higher in

older age groups More than 85 and 40 % of patients older than 55 years had positive anti-HAV and anti-HEV respectively.

Table 1 Overall hepatitis seroprevalence

	Anti-HAV	HBsAg	Anti-HCV	Anti-HDV*	Anti-HEV
Positive (%)	2996 (68.5)	387 (8.9%)	28 (0.6)	0 (0)	1456 (33.3)
Negative (%)	1375 (31.5)	3984 (91.1%)	4343 (99.4)	387 (100)	2915 (66.7)

Abbreviations: antibodies to hepatitis A virus (Anti-HAV); hepatitis B surface antigen (HBsAg); antibodies to hepatitis C virus (Anti-HCV); antibodies to hepatitis E virus (Anti-HEV)
*measurements performed among HBsAg positive subjects

Figure 1 Hepatitis seroprevalence by age group

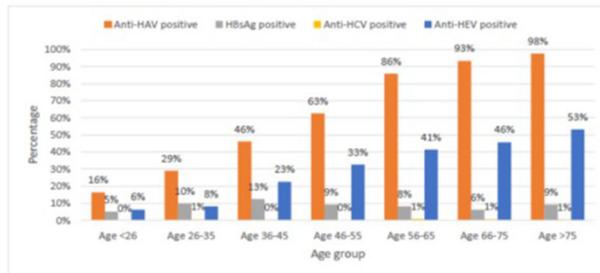
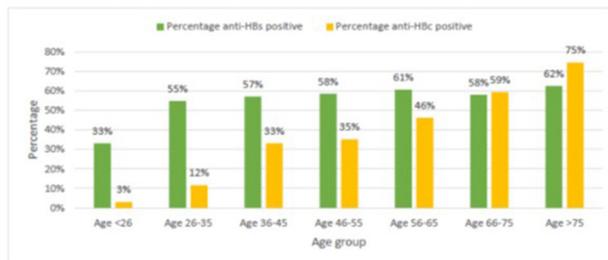


Figure 2 Percentage of positive anti-HBs and anti-HBc among HBsAg negative subjects



P-0306

HBV, HVC, HIV prevalence. Evaluation in a high risk population (1999–2014)

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Background: The Unit of Infectious Diseases since 1999 offers an anonymous screening for HIV and viral hepatitis to subjects who had a risk for HBV, HCV and HIV infection. All were evaluated at the time of exposure and later according with incubation periods.

Aims: To evaluate the prevalence of HBV, HCV and HIV among people at risk.

Methods and Results: we evaluated the 2603 subjects, tested from 1999 to 2014; 26 (0.9 %) had HBV, 97 (3.7 %) had HCV, 106 (4 %) had HIV infection. All the viral infections were evaluated for risk factors and annual prevalence.

Conclusions: HBV prevalence was slightly higher than in normal population, HCV prevalence was in adherence with normal population. Their prevalences were homogeneously distributed in the observed period, while HIV infection resulted in increased prevalence mainly related to homosexual intercourse. Mercenary Sex was the main risk factor for HBV infection, IDU was the main risk for HCV.

P-0307

Low liver disease screening and treatment rates in Mongolia: results from a physician survey

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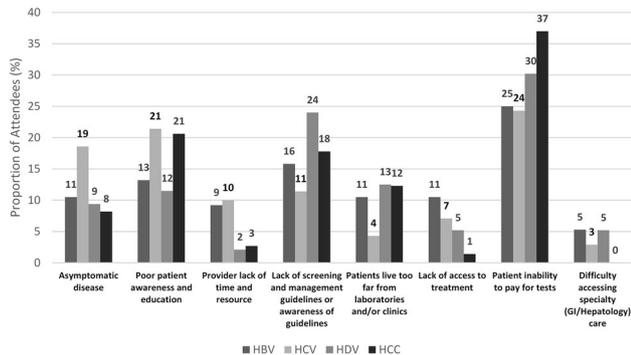
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Background: According to Globocan, Mongolia has the highest worldwide HCC incidence (78.1/100,000, 3.5× higher than China). It is unclear if screening and linkage to care for HBV, HCV, HDV, and HCC have been optimal. Our goal was to evaluate these screening rates, antiviral therapy utilization and barriers to care in Mongolia.

Methods: We conducted an anonymous survey of physicians from all major provinces who attended a 2-day CME liver symposium in Ulaanbaatar analyzing their demography, practice setting/patterns, perceptions, and proposed solutions.

Results: A total of 70–95 out of 121 (58–79 %) physician attendees responded to each question. Most were female (87 %), age <50 (79 %), sub-specialists (76 %) and practiced in urban vs. rural areas (61 vs. 39 %). Most (>80 %) noted that <50 % who need hepatitis or HCC screening receive it. The main perceived barriers to screening were inability to pay for diagnostic tests, lack of guidelines, and poor patient awareness (Figure 1). The major HCC screening barrier was also cost (37 %). Hepatitis treatment rates were low; 83 % treated HCV in <10 patients in the past year and 86 % treated HBV in <10 patients/month. Treatment barriers were multifactorial with medication cost as a principal barrier. Top proposed solutions were universal screening policies (46 %), removal of financial barriers (28 %), and provider education (20 %).

Conclusions: Physicians from all major regions of Mongolia noted low screening for viral hepatitis (<50 %) and even lower treatment rates (>80 % treated <10 patients/year for HCV <10 patients/month for HBV), and the need to remove financial barriers and increase educational efforts.



P-0308

Chronic liver disease is one of the leading causes of death in Bangladesh

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Aim: In industrialized countries, audit has become an integral part of medical care. Experience from developing countries like Bangladesh is still inadequate. This study was carried out to find out relation among some factors like age, sex, causes, diurnal variation, duration of hospital stay with death and errors in certification process.

Methods: It was a cross-sectional study conducted at Department of Medicine, Sir Salimullah Medical College and Mitford Hospital. Information of consecutive 100 deaths was collected in a predesigned clinical data sheet within half an hour of every occurrence. Necessary data were collected from hospital case records (admission registrar, case files and death certificates) using structured checklist. Patients who were brought dead were excluded from the study.

Results: Among 100 deaths, 48 % were males (48/100) and 52 % females (52/100). Within this group, 66.7 % were males and 33.3 % females. First day (within 24 h of admission) death accounted for 46 % (46/100) of all death and by the second day for 23 % (23/100). Highest underlying cause of death was cerebrovascular diseases (29 %), infectious disease contributed 20 %, chronic liver disease 13 %, malignancy 7 %, poisoning 6 %, cor pulmonale 5 %, while others 20 %.

Conclusion: In this study chronic liver disease was found to be one of the leading causes of death in our hospital and most of them occurred due to hepatic encephalopathy. Early detection of hepatic

encephalopathy and treatment is necessary to reduce hospital mortality.

P-0309

Epidemiologic study for acute hepatitis in recent years in Toyama area

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A 2011 national study about acute hepatitis in Japan showed that the incidence and constitution of acute hepatitis varied from region to region. The aim of our study was to explore the etiology and clinical manifestations of acute hepatitis diagnosed in Toyama University Hospital and its related facilities in the past 10 years. Between April 2006 and March 2015, we experienced 195 patients with acute hepatitis (not including alcoholic liver injury or drug-induced liver injury), between the age of 16 and 88 years old, 97 of which were male and 98 female. 16 were more than 65 years old. Hepatitis A accounted for 6.2 %, hepatitis B, 18.5 %, hepatitis C, 3.6 %, hepatitis E, 2.0 %, EBV, 23.1 %, CMV, 13.3 %, autoimmune hepatitis, 22.1 %, and unknown, 8.2 %, respectively. The mean ALT level was 1435 IU/L (151–10,690), the mean T-Bil. level was 5.7 mg/dl (0.4–27.4), and the mean PT activity score was 73.1 % (11–100), respectively. We experienced 15 cases of severe hepatitis, and among them, 7 patients (46.7 %) were more than 65 years old. We experienced 3 cases of fulminant hepatitis. One died, and one undertook live-donor liver transplantation, and one recovered with intensive treatment in ICU. In this study, we experienced hepatitis due to EBV and CMV more frequently than those in the 2011 national report. This may be because most of our facilities are responsible for primary care, and so have many more chances to examine people with liver dysfunction. We found the tendency that acute hepatitis was more aggravated in elderly people.

P-0310

Prevalence of hepatitis B and C and risk factors among prison inmates in Cebu, Philippines

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Background: Hepatitis B and hepatitis C (HBV and HCV) cause significant morbidity and mortality worldwide. Little is known about the existence of HBV and HCV among high risk groups like the prisoners in Filipino population. The study was conducted to determine the prevalence of HBV and HCV and risk factors among prison inmates in Cebu, Philippines.

Methods: A cross-sectional study was conducted on consenting prisoners inhabiting city and provincial jails from October 2014 to January 2015. A questionnaire was completed. Blood samples was tested for HBs Ag and Anti-HCV screening test kits. Data were analyzed using descriptive statistics. Logistic regression analysis was applied.

Results: Six hundred fifty-eight participants were enrolled. Mean age of the participants was 24 ± 0.5 years. Prevalence of HBsAg and HCV Ab were 6.8 and 27.6 %, respectively. About 2.4 % had co-

infection of HBV and HCV. A significant correlation showed history of drug inhalation (p value 0.005) and history of blood transfusion before July 1992 (p value 0.013) were likely to have HBV. City jail prisoners had a significant impact of being reactive to HCV.(p value 0.000) Being illegal drug users (p value 0.000), multiple arrests (p value 0.004), started injection in prison(p value 0.002) and having tattoo (p value 0.002) were more likely to have HCV.

Conclusions: This study showed high prevalence of HCV than HBV among prison inmates. Usage of injecting drugs, unsterilized syringes, tattoo injections and multiple incarcerations were important risk factors. Our results indicated the importance of policies to prevent transmission of HCV during and following incarceration.

Table 1. Distribution of the Demographic Characteristics of Prison Inmates

Characteristics	Total (n, %)	HBV (n, %)	HCV (n, %)
Jail:			
Provincial	328 (49.8)	20 (3.0)	28 (4.3)
City	330 (50.2)	25 (3.8)	153 (23.3)
Age:			
18 – 24	150 (22.8)	8 (1.2)	46 (7.0)
25 – 30	151 (22.9)	6 (0.9)	54 (8.2)
31 – 35	105 (16.0)	9 (1.4)	28 (4.3)
36 – 45	139 (21.1)	14 (2.1)	40 (6.1)
46 – 55	72 (10.9)	6 (0.9)	12 (1.8)
56 – 60	20 (3.0)	1 (0.2)	1 (0.2)
>60	21 (3.2)	1 (0.2)	-
Sex:			
Male	556 (84.5)	40(6.1)	163 (24.8)
Female	102 (15.5)	5 (0.8)	18 (2.7)
Marital Status:			
Single	447 (67.9)	30 (4.6)	139 (21.1)
Married	195 (29.6)	14 (2.1)	40 (6.1)
Widowed/Widower	16 (2.4)	1 (0.2)	2 (0.3)
Number of Marriage:			
None	445 (67.6)	30 (4.6)	139 (21.1)
Once	207 (31.5)	15 (2.3)	41 (6.2)
Twice	4 (0.6)	-	1 (0.2)
Thrice or more	2 (0.3)	-	-
Education:			
Illiterate	10 (1.5)	1 (0.2)	-
Primary	4 (0.6)	1 (0.2)	-
Elementary	227 (34.5)	19 (2.9)	58 (8.8)
High School	321 (48.8)	18 (2.7)	102 (15.5)
College	96 (14.6)	6 (0.9)	21 (3.2)
Crime Type:			
Crime against persons	164 (24.9)	9 (1.4)	26 (4.0)
Crime under special penal laws-committed against the state: drugs, drug paraphernalia	287 (43.6)	22 (3.3)	83 (12.6)
Crime under special penal laws-committed against the State: firearms, gun band, gambling	53 (8.1)	6 (0.9)	20 (3.0)
Crime under special penal laws-committed against persons	39 (5.9)	1 (0.2)	7 (1.1)
Crime against property	111 (16.9)	5 (0.8)	43 (6.5)
Crime against chastity	1 (0.2)	1 (0.2)	-
Crime against security and liberty	3 (0.5)	1 (0.2)	2 (0.3)
Number of Arrest:			
Once	514 (78.1)	35 (5.3)	96 (14.6)
2 – 3	116 (17.6)	8 (1.2)	64 (9.7)
4 or more	28 (4.3)	2 (0.3)	21 (3.2)
Duration of Imprisonment:			
<12 months	290 (44.1)	24 (3.6)	88(13.4)
1 – 1.99 years	142 (21.6)	7 (1.1)	29(4.4)
2 – 2.99 years	73 (11.1)	5 (0.8)	28 (4.3)
3 – 4.99 years	89 (13.5)	4 (0.6)	22(3.3)
5 – 7.99 years	39 (5.9)	2 (0.3)	9 (1.4)
8 – 10 years	15 (2.3)	1 (0.2)	2 (0.3)
More than 10 years	10 (1.5)	2 (0.3)	3(0.5)

Table 2. Distribution of the Risk Factors for HBV and HCV among Prison Inmates

Factors	Total (n, %)	HBV (n, %)	HCV (n, %)
History of Use of Illegal Drugs:			
Yes	495 (75.2)	35 (5.3)	174 (26.4)
No	163 (24.8)	10 (1.5)	7 (1.1)
Routes of Drug Use:			
Inhalation	321 (64.8)	20 (4.0)	56 (11.3)
Injection	4 (0.8)	-	-
Ingestion	5 (1.0)	-	2 (0.4)
Inhalation and Injection	101 (20.4)	10 (2.0)	87 (17.6)
Inhalation and Ingestion	35 (97.1)	2 (0.4)	7 (1.4)
Injection and Ingestion	2 (0.4)	-	1 (0.2)
Inhalation, Injection and Ingestion	27 (5.5)	3 (0.6)	21 (4.2)
Drug inhalation use in the past 12 months inside the prison:			
Yes	198 (30.1)	22 (3.3)	74 (11.2)
No	460 (69.9)	23 (3.5)	107 (16.3)
Start of injection inside the prison:			
Yes	54 (8.2)	5 (0.8)	38 (5.8)
No	604 (91.8)	40 (6.1)	143 (21.7)
Sharing of needles inside the prison:			
Yes	159 (24.2)	7 (1.1)	63 (9.6)
No	499 (75.8)	38 (5.8)	118 (17.9)
Persons notified that they received blood from a donor who later tested positive for Hepatitis B or C infection:			
Yes	2 (0.3)	-	1 (0.2)
No	656 (99.7)	45(6.8)	180 (27.4)
Persons who received a transfusion of blood/ blood components before July 1992:			
Yes	16 (2.4)	4 (0.6)	3 (0.5)
No	642(97.6)	41 (6.2)	178 (27.1)
Persons born to HBV or HCV positive mother:			
Yes	4 (0.6)	-	1 (0.2)
No	654 (99.4)	45 (6.8)	180 (27.4)
Persons who have history of sexually transmitted disease			
Yes	97 (14.7)	7 (1.1)	47 (7.1)
No	561 (85.3)	38 (5.8)	134 (20.4)
Persons who has greater than 20 sexual partners in his/her lifetime or other high risk sexual behavior:			
Yes	223 (33.9)	22 (3.3)	87 (13.2)
No	435 (66.1)	23 (3.5)	94 (14.3)
Persons who have tattoo:			
Yes	495 (75.2)	33 (5.0)	162 (24.6)
No	163 (24.8)	12 (1.8)	19 (2.9)
Persons who have body piercing:			
Yes	459 (69.8)	30 (4.6)	136 (20.7)
No	199 (30.2)	15 (2.3)	45 (6.8)
Persons born during 1945-1965			
Yes	67 (10.2)	6 (0.9)	6 (0.9)
No	591 (89.8)	39 (5.9)	175 (26.6)

Table 3. Distribution of Co-Infections of Hepatitis B and C among Prison Inmates

HBV	HCV				Total	
	Negative		Positive		n	%
	n	%	n	%	n	%
Negative	448	68.1	165	25.1	613	93.2
Positive	29	4.4	16	2.4	45	6.8
Total	477	72.5	181	27.5	658	100.0

Table 4. Multiple Logistic Regressions of Risk Factors and Demographic Characteristics for HBV

	B	S.E.	Wald	df	P value	Odds	95% CI for Odds	
Drug inhalation in the past 12 months inside the prison	0.936	0.330	8.061	1	0.005	2.551	1.336	4.869
Transfusion of blood/blood components before July 1992	1.623	0.652	6.201	1	0.013	5.067	1.413	18.171

Table 5. Multiple Logistic Regressions of Risk Factors and Demographic Characteristics for HCV

	B	S.E.	Wald	df	P value	Odds	95% CI for Odds	
Jail	1.719	0.246	48.922	1	0.000	5.578	3.446	9.030
Arrested once	-1.395	0.487	8.208	1	0.004	0.248	0.096	0.644
Illegaldrugs user	1.682	0.418	16.186	1	0.000	5.378	2.370	12.206
Started injection inside the prison	1.098	0.350	9.850	1	0.002	2.997	1.510	5.948
Have tattoo	0.935	0.296	9.969	1	0.002	2.547	1.426	4.552

P-0311

Seroprevalence of hepatitis A and hepatitis B

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Turkey is an intermediate endemic country for hepatitis B and hepatitis A. Hepatitis B vaccine was implemented in 1998 and hepatitis A vaccine was implemented in 2012 to national childhood immunization programme. This study aimed to determine the seroprevalence of hepatitis A and B in adult population in our region.

Materials and methods: Patients admitted to our department for screening tests with any reason were included in the study. The study was conducted at between September 2014 and September 2015. The patients diagnosed with chronic hepatitis B infection previously were excluded from the study. All the patients were screened for HBsAg, anti-HBc IgG, anti-HBs, anti-HAV IgG by using ELISA. Both of the positivity of anti-HBc IgG and anti-HBs were considered as naturally acquired immunity whereas merely anti-HBs positivity was the result of vaccination. Test results were evaluated according to the age groups.

Results: Totally 580 patients (309 females, 271 males) were screened. Vaccine coverage of hepatitis B was high with a rate of 84.9 % in <20 years old group. Encountering with hepatitis B and A virus was increasing with the age. All the results were shown in Table.

Conclusion: It is needed to build control programmes targeting adults for hepatitis B and young adults for hepatitis A.

Table.1. Hepatitis A and B seroprevalance according to the age groups

Age groups	Hepatitis B			HAV IgG seropositivity N %
	Vaccinated N (%)	Naturally immune N (%)	Seronegative N %	
<20 yrs. (172)	146 (84.9%)	3 (1.7%)	23 (13.4%)	33 (18.2%)
20-29 yrs. (178)	88 (49.4%)	13 (7.3%)	77 (43.3%)	85 (47.7%)
30-39 yrs. (102)	28 (27.4%)	21 (20.7%)	53 (51.9%)	77 (75.5%)
40-49 yrs. (77)	13 (16.9%)	29 (37.7%)	35 (45.6%)	76 (98.7%)
50-59 yrs. (33)	5 (15.1%)	13 (39.4%)	15 (45.5%)	33 (100%)
>60 yrs. (18)	5 (27.7%)	11 (61.1%)	2 (11.1%)	18 (100%)

P-0312

Hepatitis B vaccination among dental practitioners in Sri Lanka

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Background: Hepatitis B is one of the important blood born infections encountered in clinical practice. Prevention of hepatitis B transmission is a major component in any infection control program. Vaccination considered being the most effective method of prevention and therefore it is recommended for clinical health care providers. Effectiveness of vaccination depends on the seroconversion and evaluation of immune status following vaccination is recommended to ensure the protection. The objective of this study is to evaluate the improvement of hepatitis B vaccination and post vaccination immune status among Sri Lankan dental health care providers. **Materials and Methods:** This is a questionnaire based descriptive study. Randomly selected dental practitioners in Sri Lanka were included in the study. Vaccination rate and evaluation of immune status were assessed in 1997 and 2014.

Results: in 1997

- Number of dental practitioners (average): 600
- Number of practitioners selected: 300
- Number of responders: 151
- Vaccination rate: 80 %
- Evaluation of immunity: 7.7 %
- Adequate immunity: data not available

- in 2014
- Number of dental practitioners (average): 1200
- Number of practitioners selected: 600
- Number of responders: 181
- Vaccination rate: 93.9 %
- Evaluation of immunity: 18.2 %
- Adequate immunity: 90.3 %

Conclusions: There is a significant improvement in vaccination though it needs to be improved further. Evaluation of immune status remains unsatisfactory and this need to be encouraged as significant number of immune failures are seen. Vaccination against hepatitis B and evaluation of post vaccination immune status should be encouraged further to reach evidence based standards and to achieve safe clinical practice.

P-0313

Impact of universal hepatitis B vaccination into newborn as part of Thailand EPI program since 1988

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Universal HB vaccination in newborns had been introduced as part of Thai EPI since 1988. Our previous studies showed that children and adolescents who were born after the HB vaccine implementation have a carrier rate of less than 1 % compared with 5–6 % carrier rate among them before the HB vaccine program. In 2014, we did the national hepatitis B serosurveys among 5581 subjects in the different parts of the country. The long-term immunogenicity and impact of universal hepatitis B vaccination into newborn as part of EPI program for more than 20 years were evaluated by HBsAg, antiHBc and antiHBs. HBV carriers of children and young adults, who were born after universal HB vaccination, were markedly reduced. The carrier rate among the age groups of <5, 5–10, 10–20, 20–30, 30–40, 40–50 and >50 years old was 0.1, 0.3, 3.1, 3.8, 4.7 and 6.0 % respectively. HBV infection by mean of detectable antiHBc was also drastically declined in the young population. Base on the total Thai population with the government data recorded; we estimated that the total number of HBV carriers amounted to 2.22 million cases or 3.48 % of the total populations. Most of them were in adult age group. HB vaccine represents the first vaccine shown to be effective in preventing the occurrence of chronic liver diseases including HCC. It is believed that HB vaccine will facilitate the implementation of universal vaccination campaigns and thus contribute to the control and possible to the eventual eradication of this disease.

P-0314

Immunogenicity and efficacy of a 10µg recombinant yeast-derived hepatitis B vaccine in newborns

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Background: To evaluate immunogenicity and efficacy of a 10µg recombinant *Saccharomyces cerevisiae*-derived hepatitis B vaccine (Kangtai Biological Products Co. Ltd, Shenzhen, China) (Hep-KSC) in newborns.

Methods: Overall 1197 infants born to mothers negative for HBV markers (NM group) and 534 born to HBsAg-positive mothers (PM Group) were enrolled. Infants in NM group were given 10µg Hep-KSC, 10µg Enderix-B or 5µg Hep-KSC and those in PM group received 10µg Hep-KSC or 10µg Enderix-B at 0, 1 and 6 months, with an additional 200 IU HBIG at birth for the latter.

Results: For NM Group, 10µg Hep-KSC paralleled 10µg Enderix-B but outperformed 5 µg Hep-KSC regarding seroprotective rate (95.06 vs 94.83 vs 89.67 %, $p = 0.0077$) and anti-HBs geometric mean concentration (GMC) (798.87 vs 790.16 vs 242.04 mIU/ml, $p < 0.0001$) at 7 months. The proportion of infants with anti-HBs greater than 1000 mIU/ml was higher in 10µg Hep-KSC than 5µg Hep-KSC group (45.77 vs 11.93 %, $p < 0.0001$) at 7 and 12 months. For PM Group, the HBsAg positivity rate in 10µg Hep-KSC and 10µg Enderix-B group was 1.60 and 4.27 % at 7 months, respectively. In 10µg Hep-KSC group, 93.61 and 91.29 % achieved seroprotection at 7 and 12 months, respectively, and correspondingly 90.24 and 86.96 % in 10µg Enderix-B group. The anti-HBs GMC was comparable between 10µg Hep-KSC and 10µg Enderix-B group at 7 and 12 months (575.31 vs 559.64 mIU/ml; 265.79 vs 264.48 mIU/ml).

Conclusions: 10µg Hep-KSC might be appropriate for neonatal immunization with good immunogenicity and efficacy, especially for infants born to HBsAg-positive mothers.

P-0315

Similar intrahepatic, serum virus-load and HCC in hepatitis B genotype B&C with advanced fibrosis

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Introduction: Hepatitis B is leading cause of liver related morbidity in Asia with predominant genotypes B and C in east-Asia. Serum, intrahepatic viral-markers and long-term follow-up data of genotypes(GT) B and C in patients with biopsy-proven advanced fibrosis is sparse.

Aims: To compare serum and intrahepatic viral markers, development of Hepatocellular carcinoma (HCC) in GT B and C in patients with advanced fibrosis (Ishak ≥ 4).

Patients and Methods: 63 Treatment-naive patients identified with advanced fibrosis on liver-biopsy done between 1998 and 2000 at Singapore-General-Hospital. FFPE tissue was available for 59 patients and serum for 42 patients. HBV DNA was quantified in serum and liver while qHbsAg quantified in serum. Patients were followed-up for HCC development.

Observation: The mean age was 65.0±10.7 years, with 77.7 % males. 43 patients were GT-B, 19 were GT-C and one had both genotype B&C. Mean follow-up was 13.5 years. The sHBV-DNA was 6.30 ± 1.4 and 6.48 ± 1.25 log IU/ml, sHbsAg was 3.39 ± 0.67 and 3.44 ± 0.55 log IU/ml and intrahepatic HBV-DNA was 2.66 ± 4.2 copies/cell and 1.54 ± 3.06 copies/cell in the genotypes B and C respectively ($p > 0.1$ in all). Complete cirrhosis (Ishak 6) was present in 47.6 %, Ishak-5 fibrosis in 33.3 % and Ishak-4 fibrosis in 19 % at recruitment. On follow-up HCC developed in 3/19 in GT-B and 8/43 in GT-C ($p = 0.86$) advanced age and cirrhosis were significant factors for development of HCC.

Conclusion: No difference serum HBV-DNA, serum HBsAg or intrahepatic HBV-DNA was seen between the two genotypes. Development of HCC was similar over long-term follow-up in two genotypes.

P-0316

High prevalence of HBV infection, genotypes H, F1b and G in socially vulnerable Mexican risk groups

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Background: Hepatitis B virus (HBV) infection among patients with human immunodeficiency virus (HIV) and hepatitis C virus (HCV), and rural Native people (RNP) may be underestimated in Mexico, a country apparently of low endemicity.

Aim: To assess the prevalence of serological markers, genotypes (GTs) and risk factors (RFs) for HBV infection among socially vulnerable risk groups in West Mexico.

Methods: A total of 718 patients were included: HIV (n = 243), HCV (n = 289), urban population with clinical hepatitis (UPCH) (n = 87), 55 RNP (NPOaxaca) and 44 RNP (NPMichoacan). Demographic, clinical and RFs data were collected. HBsAg and anti-HBc were tested by ELISA. Viral load (VL) and HBV GTs were assessed by COBAS and direct DNA sequencing, respectively.

Results: All patients had very low education and income. The prevalence of HBV was 34 % (234/695) and for each group: VIH (54 %), UPCH (41 %), HCV (21 %), NPOaxaca (25 %), NPMichoacan (9 %). Anti-HBc was twofold higher than HBsAg (30 vs 16 %, p < 0.001). In 88 of 113 HBsAg+ patients, 66 had a detectable VL (Median, 254 IU/mL). Among 31 GTs detected, H was 58 %, F1b (16 %), A2 (13 %), G (10 %), D4 (3 %). RFs were surgery (UPCH and NPOaxaca) and sexual promiscuity (UPCH). The RF associated with HBV/HCV or HIV coinfection was injecting drug use (OR = 2.93, 95 % CI 1.05–8.09; OR = 2.68, 95 % CI 1.08–6.61, p < 0.05, respectively); being single in HBV/HCV (OR = 5.84, 95 % CI 1.91–17.08) and MSM in HBV/HIV (OR = 2.64, 95 % CI 1.39–5.04), (p < 0.05).

Conclusions: Among socially vulnerable risk groups, a high prevalence of HBV infection was detected by testing both markers, VL was low, GTs and RFs were distinct between groups.

P-0317

Sequencing HBV polymerase and surface genes circulating in a Bangladeshi cohort

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Aim: Hepatitis B virus (HBV) shows variability due to the kinetics of viral production, error prone reverse transcriptase, long term antiviral therapy and vaccination, leading to the emergence of many

genotypes, subtypes and HBV variants. The present study aimed to determine HBV genotypes, subtypes and mutations in the polymerase and surface genes associated with antiviral resistance and hepatitis B surface antigen (HBsAg) mutations among untreated and treated Bangladeshi chronic hepatitis B (CHB) patients.

Methods: A total of 29 patients were included in this study from the Department of Virology, Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh. HBV DNA sequencing was performed by Sanger method.

Results: Our results showed genotype C in 17/29 (58.6 %), genotype D in 10/29 (34.5 %) and genotype A in 2/29 (6.9 %) cases. The subtypes observed were adr_q+ in 17/29 (58.6 %), ayw₃ in 8/29 (27.6 %), adw₂ in 2/29 (6.9 %) and ayw₂ in 2/29 (6.9 %). The frequency of antiviral mutations in the untreated group was 1/15 (6.7 %), and the pattern was rtQ215H, while in the treated group this frequency was 3/14 (21.4 %) and the patterns were rtA181V, rtM204V + rtL180M. Moreover, the frequency of HBsAg mutations in the untreated group was 7/15 (46.7 %) and the patterns were sT118V + sP127T + sA128V, sI110L + sP127T, sY100F, sT114S and sT140S, whereas in the treated group it was 5/14 (35.7 %) and the patterns were sT118V + sP127T + sA128V and sI126V.

Conclusion: Our study concluded that HBV genotype C was predominant among Bangladeshi CHB patients followed by genotype D, while genotype A was the least dominant. Antiviral resistance and HBsAg mutations of HBV were evidently present in Bangladesh and need further evaluation.

P-0318

HBsAg quantity for monitoring of hepatitis B virus infection in inactive carriers

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187 HBsAg positive patients were studied. Patients with additional etiological factors of liver damage and patients who previously received antivirals were excluded. Inactive carriers (IC) criteria were: HBsAg presence, normal TA level during 1 year at a 3-fold measurement, DNAHBV level isn’t more than 2000 IU/ml, elastography result isn’t more than 7 kPa. The IC were 104 people (55 %), patients with HBe-negative chronic hepatitis B were 52 (28 %), patients with HDV-22 (12 %), patients with HBe positive chronic hepatitis B-7 (4 %); immunotolerant patients-2 (1 %). 66 patients (IC) were followed every 6–12 months. Median follow-up-2.4 years. If patient’s TA and HBV DNA remained within criteria of IC they were designated as “stable patients”; patients who were identified HBV DNA fluctuations (sometimes accompanied by the rise of TA) exceeding the criteria of IC were conventionally called “patients with HBV reactivation”. The median level of ALT in “stable patients” group was 19 U/l, and in “patients with HBV reactivation” group was 17.85 U/l, in “stable patients” group median level of DNA was 166 IU/ml and 455 IU/ml in “patients with of HBV reactivation” group, levels of fibrosis didn’t differ and were 5.35 kPa in “patients with reactivation of HBV infection” and 4.5 kPa in “stable patients” group (p > 0.05). HBsAg level in the “stable patients” group was 647.29 U/ml, and in the “patients with HBV reactivation” was 3393.21 U/ml (p < 0.05). Patients with baseline HBsAg level less than 1000 U/ml may occur less frequently than once per year. Patients with HBsAg level over 2000 U/ml requires dynamic observation because of risk of a possible chronic HBV-infection reactivation.

P-0319

HBsAg analysis using the highly sensitive lumipulse HBsAg-HQ assay in patients with liver disease

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Background: The sensitivity of the Lumipulse HBsAg-HQ assay (HQ assay; Fujirebio, Japan) that has been improved with a measurement limit of 0.005 IU/mL, compared with 0.05 IU/mL for the Architect HBsAg assay (QT assay; Abbot, IL, USA). We aimed to assess the prevalence and characteristics of HBsAg-positive cases by performing the HQ assay, using stored serum samples of patients with liver disease who tested negative for HBsAg in the QT assay.

Methods: In total, 4950 patients with liver disease [chronic hepatitis C infection 2770, non-alcoholic fatty liver disease in 457, liver tumor in 488, alcoholic liver disease in 419, autoimmune liver disease in 340, and acute liver injury in 476 patients] who visited our hospital and tested negative for HBsAg in the QT assay were evaluated. Their mean age was 62 years, and 2600 of them were men. HBsAb and hepatitis B core antibody (HBcAb) were measured by using Lumipulse anti-HBs and Lumipulse anti-HBc, respectively.

Results: Of the 4950 patients, 40 (0.8 %) tested positive in the HQ assay. In 16 patients with positive results whose samples were available for longitudinal measurement by HQ assay, HBsAg positivity rates were 63 % at 1 year, 56 % at 2 years, 40 % at 3 years, 40 % at 4 years, and 40 % at 5 years, as determined by the Kaplan–Meier method.

Conclusion: Of the 4950 patients with negative QT assay results, 40 (0.8 %) tested positive for HBsAg in the HQ assay. The HBsAg positivity rate in the HQ assay decreased to approximately 40 % in 5 years.

P-0320

Evaluation of serum qHBsAg levels and liver biopsy in inactive hepatitis B carriers

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Introduction: In our study, we aimed to evaluate serum levels of qHBsAg in inactive HBV carriers and HBeAg negative chronic HBV patients and to compare this with liver biopsies.

Materials and Methods: Seventy one patients were included in the study. Serum HBsAg levels were quantified using the Abbott ARCHITECT assay (Abbott Diagnostics, Germany) (the sensitivity was 0.05–250 IU/mL). The levels of HBV DNA were quantified using the COBASTaqMan (Roche Molecular Systems, USA), with a lower detection limit of 20 IU/mL. Liver biopsy specimens were assessed according to the modified Ishak scoring. Patients were performed two groups according to do HBV DNA levels. Group 1 HBV DNA levels <2000 IU/ml, group 2 HBV DNA levels >2000 IU/ml.

Results: The mean age was 42.3 ± 11.2 years and 46 % of them were male. All patients had genotype D and anti HBe positive. The mean histological activity index and fibrosis score were 6.2 ± 1.9 and 2.0 ± 1.2 respectively. The mean qHBsAg levels was 4919 ±

4692 IU/ml. The mean HBV DNA levels was 7233 ± 14,327 IU/ml. Comparison of two groups is shown in table.

Conclusion: The serum qHBsAg titer is positively correlated with HBV DNA levels but not fibrosis score Group 1 HBV DNA <2000 IU/ml Group 2 HBV DNA >2000 IU/ml Number of patients 40 31 age (years) 45.9 ± 9.7 37.7 ± 11.4 HBV DNA levels (IU/ml) 502 ± 518 15,919 ± 18,446 ALT (U/L) 20.3 ± 8.0 28.7 ± 16.6 Quantitate HBsAg levels (IU/ml) 2331 ± 1465 5219 ± 5880 Histological activity index (Ishak) 5.9 ± 2.2 5.9 ± 1.5 Fibrosis (Ishak) 1.9 ± 1.4 1.8 ± 0.9

Table: Comparison of two groups according to do HBV DNA levels.

P-0321

Distribution of quantitative HBsAg level according to serum HBV DNA level in chronic hepatitis B

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Recently, assessment of HBsAg level has been increasingly emphasized in clinical settings. Therefore, we investigated the distribution of HBsAg levels in combination with serum HBV DNA level in chronic hepatitis B (CHB). A total of chronic hepatitis B patients who had checked HBsAg quantification and serum HBV DNA level together between and April 2013 and January 2015 were included in this study. Liver biochemistry, HBV serological markers, serum HBV DNA and HBsAg titer were checked. Among 1824 patients, 1485 (81.4 %) patients had treated with nucleos(t)ide analogues (Group A) and 339 (18.6 %) patients did not received any antiviral therapy (Group B). Among the Group A patients with HBV DNA more than 20,000 IU/mL, the percentage of HBsAg titer with less than 102, 102–103, 103–104 and more than 104 IU/mL were 12.8, 33.8, 44.4 and 9.0 %. Among the patient with HBV DNA less than 20 IU/mL, the most common of HBs titer was 103–104 IU/mL (594/1196, 49.7 %). The mean value of HBsAg decreased during antiviral therapy and it was significantly lower than those at baseline. HBeAg seroclearance and/or biochemical response was associated with lower HBsAg level. In Group B, the percentage of HBs titer with less than 102, 102–103, 103–104, more than 104 IU/mL were 26.1, 27.8, 28.8 and 17.3 %. Distribution of HBsAg levels and HBV DNA levels differ in patients between with and without antiviral therapy. In addition, even in patients with HBV DNA less than 20 IU/mL, the measurement of HBsAg level would be anticipated to monitor antiviral efficacy.

P-0322

Detection of HBV surface antigen mutations in patients with chronic HBV by ELECSYS HBsAg II assay

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Background: There is a paucity of data regarding the capacity of routine HBsAg assays to detect HBsAg with naturally occurring mutations in the HBsAg “a” determinant region.

Methods: Prospectively collected samples (n = 450) obtained from chronic HBV infected patients in Beijing (n = 300) and Guangzhou (n = 150) were included in the study between January 2014 and October 2015. Samples were (a) analyzed for the presence of the HBsAg using the Roche ELECSYS HBsAg II Qualitative assay and (b) sequenced for HBsAg mutations.

Results: Of the 450 samples, 284 (63.1 %) samples had at least one of 193 different type amino-acid substitutions (distinct HBsAg mutations) in the “a” determinant HBsAg region, with a total of 664 mutations identified, located both inside (n = 384) and outside the HBsAg “a” determinant region (n = 280). The remaining 166 samples did not bear mutations in the HBsAg. All 450 samples were positive for HBsAg using the ELECSYS HBsAg II Qualitative assay, with a sensitivity of 100 %, with lower 95 % confidence limit of 99.18 %. The complete spectrum of 664 mutations with common immune escape associated mutations (P120T, G145R, M133L, M133T, Q129H, G130N, S143L, T126S, D144A and T131I) was consistently detected by the ELECSYS HBsAg II Qualitative assay.

Conclusions: Our results indicate that the capacity of the ELECSYS HBsAg II Qualitative assay to detect chronic HBV infection is not compromised by the known spectrum of naturally occurring HBsAg mutations and suitable for routine diagnostic use in patients with chronic HBV from China.

P-0323

Dynamic changes of HBsAg in HBV patients: genotype C had a greater HBsAg decline than genotype B

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Aim: Dynamic changes of serum hepatitis B surface antigen (HBsAg) levels in chronic hepatitis B patients with genotype B or C after different treatment have not been well-studied.

Methods: The patients were randomized received entecavir (ETV), ETV combination PEG-interferon or EVT combination thymosin-a treatment for at least 2 years. HBV genotype was determined at baseline. Liver biochemistry, HBV serological markers, serum HBV DNA and HBsAg titers were determined at baseline, and treatment 1 year.

Results: Total of 314 patients (69 genotype B) were recruited. After 1 year treatment, genotype B patients had a significantly greater HBsAg decline compared with genotype C [log₁₀ IU/mL, 0.357 (−0.508, 5.731) vs. 0.099 (−2.022, 4.717)]. Genotype B patients, when compared to genotype C patients, HBsAg titers had a higher proportion of HBsAg reduction more than 1 log₁₀ IU/mL (15.9 and 6.5 % respectively). Further study we perform subgroup analysis based therapy, among the patients treated with EVT (69), EVT combination PEG-interferon (245) or EVT combination thymosin-a (130), the decreasing of HBsAg titers in genotype B patients were significantly greater than genotype C [log₁₀ IU/mL, 0.195 (−0.508, 1.569) vs. 0.0595 (−2.022, 1.421); 0.804 (−0.011, 5.73) vs. 0.284 (−0.624, 4.717); 0.134 (−0.128, 1.058) vs. 0.0656 (−1.089, 1.335), respectively].

Conclusion: Chronic hepatitis B patients with genotype C had a greater HBsAg decline than genotype B after treatment 1 year

P-0324

Plasma adiponectin levels and chronic HBV infection characteristics and milestones

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Introduction: Our previous nested-case-control study found that elevated plasma adiponectin is independently associated with an increased liver cirrhosis and HCC risk in HBV carriers.

Materials and Methods: Plasma adiponectin was determined from the biosamples collected at baseline from REVEAL-HBV cohort to investigate the association between adiponectin and baseline HBV infection characteristics including HBeAg seropositive, high HBV viral load and HBsAg titers. Subsequent HBeAg, HBsAg seroclearance as well as HBV DNA undetectability, and ultimately the development of cirrhosis, HCC and liver related death during follow-up were investigated.

Results: Among 3931 HBsAg(+)/anti-HCV(−) participants, 3692 had sufficient biosamples left for adiponectin assay. Up to 2011, with a mean 18 years follow-up, there were 306 newly diagnosed HCC and 285 died from liver related causes. Elevated adiponectin was associated with higher chance of HBeAg seropositive, high HBV viral load (>200,000 IU/mL) and high HBsAg titers (>1000 IU/mL) in a dose-response manner, with a two-fold increased risk (OR = 2.21, 95 % CI 1.52–3.22; OR = 2.08, 95 % CI 1.45–3.00 and OR = 1.91, 95 % CI 1.45–2.50) for Q5 vs. Q1, respectively. Those with the highest quintile had a lower chance of achieving HBsAg (HR = 0.48, 95 % CI 0.27–0.85), HBeAg (HR = 0.71, 95 % CI 0.50–1.00) seroclearance, and HBVDNA undetectability (HR = 0.61, 95 % CI 0.43–0.88) during follow-up, and higher chance of developing liver cirrhosis (HR = 2.73, 95 % CI 1.87–3.98), HCC (HR = 2.51, 95 % CI 1.52–4.15), and eventually died from liver related causes (HR = 2.40, 95 % CI 1.49–3.85).

Conclusion: We found that elevated adiponectin is consistently associated with all important chronic HBV infection milestones towards disease progression.

P-0325

HBsAg (+) patients distribution, clinical and demographic characteristics in Turkey Sanliurfa region

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Aim: HBsAg positivity is approximately 4 % in Turkey. It was aimed to investigate clinical distribution and demographic characteristics of HBsAg (+) patients living in Sanliurfa.

Methods: HBsAg (+) patients, December 2011–April 2012, were differentially diagnosed as inactive carriers, chronic hepatitis B (CHB), cirrhosis, chronic delta hepatitis, and delta cirrhosis.

Results: Of 296 patients with positive HBsAg, 152 (51 %) were males, mean age 38.8 (range 15–74). Of patients, 205 (69 %) were inactive HBV carriers; 44 (16 %) had CHB; 30 (10 %) had cirrhosis. Anti-delta was positive in 17 (5 %) of patients, and 5 of them were at cirrhotic stage. Seven of patients were pregnant. HCC was diagnosed in two of patients. Antiviral treatment was employed in 71 patients with chronic hepatitis or cirrhosis stage related to HBV or delta hepatitis. Antiviral treatment drugs and patient numbers were tenofovir, entecavir, lamivudine, pegylated interferon; 39 (55 %), 12 (17 %), 7 (10 %), 13 (18 %), respectively. Tenofovir was given in the 3rd trimester in 3 out of 7 pregnant women because of high viral load. **Conclusion:** Of HBsAg (+) patients in Sanliurfa region, three fourth of them are inactive HBV carriers; one fourth of them have chronic hepatitis or are at the cirrhotic stage. Among HBsAg (+) patients, incidence of chronic delta hepatitis is 5 %. Nearly all patients with chronic hepatitis or cirrhosis have received/are receiving antiviral treatment, and tenofovir is the most commonly used agent.

P-0326

HBsAg seroclearance after treatment-induced HBeAg seroclearance in chronic hepatitis B patients

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Aim: To explore the factors associated with hepatitis B surface Antigen (HBsAg) seroclearance after treatment-induced hepatitis B e-Antigen (HBeAg) seroclearance in chronic hepatitis B (CHB) patients based on the real-world clinical database.

Methods: A retrospective cohort study was conducted in 1072 CHB patients with treatment-induced HBeAg seroclearance between February 14, 2008 and December 31, 2012 in Beijing. The predictive accuracy of HBsAg level at HBeAg seroclearance was evaluated by time-dependent receiver operating characteristic curve. Cox regression model was used to explore the associated factors of HBsAg seroclearance.

Results: After 1120 person-years' follow-up of 1072 CHB patients, 25 patients underwent HBsAg seroclearance. The predictive accuracy of HBsAg titers at HBeAg seroclearance was 89.1, 92.4 %, 91.6 and 81.6 % for HBsAg seroclearance at 6, 12, 24 and 36 months, with the optimal cut-offs of 16.6 IU/ml for the 6-month prediction and 2096.0 IU/ml for the other 3 time points. After adjusting gender, age at HBeAg seroclearance, HBV DNA and status of stable anti-HBe, HBsAg levels at HBeAg seroclearance were still observed to be associated with HBsAg clearance. Compared with the patients with HBsAg level ≥ 2096.0 IU/mL at HBeAg seroclearance, the hazard ratio (HR) of HBsAg clearance for those with HBsAg level < 16.6 IU/mL was 155.2 (95 % CI 27.6–871.3, $P < 0.0001$), and those with HBsAg level between 16.6 and 2096.0 IU/mL was 14.6 (95 % CI 4.2–101.2, $P = 0.0002$).

Conclusion: Lower HBsAg level at HBeAg seroclearance was associated with higher likelihood of treatment-induced HBsAg seroclearance within 2 years.

P-0327

High rate of hepatitis B surface antigen mutations in Chinese patients with chronic HBV infection

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Background: Immunodominant “a” determinant region (amino acids 120–147) mutations are clinically important as they are associated with clinical complications and HBsAg false negative diagnostic test results. The objective of this study was to explore the prevalence of HBV surface antigen gene mutations in chronic HBV infected patients from China.

Methods: A total of 300 samples from patients with chronic HBV infection obtained at Youan Hospital, Capital Medical University Beijing, were characterized for immunodominant “a” determinant region mutations using next generation ultra-deep sequencing. To explore potential location-dependent aspects of the mutations, the immunodominant “a” determinant region was sub-grouped into the “mini loop (amino acid positions 120–123), the “first loop” (aa 124–137), and the “second loop” (aa 138–147), and the mutations rates were compared between the three subdomains.

Results: The HBV genotype distribution of 281 successfully sequenced patient samples was: B = 67 (23.8 %), C = 212 (75.4 %), D = 2 (0.71 %). Only 19 of the 300 samples could not be genotyped or sequenced to completeness. We found that 249 (62.5 %) out of 398 different mutations in the major hydrophilic region (MHR) were located in the immunodominant “a” determinant region. The first loop subdomain displayed the highest mutation frequency (79.5 %) followed by the second loop (13.2 %) and the mini loop (7.2 %), respectively. The well-known vaccine and immune escape associated mutation G145R was identified in 13 (7.4 %) of the 175 mutated samples.

Conclusions: The frequencies of mutations in the immunodominant MHR “a” determinant region and the vaccine escape associated G145R mutation are markedly higher than previously reported.

P-0328

Trace amount of HBsAg in samples for pre-surgery testing was detected using an ultra-sensitive assay

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Objective: We aimed to evaluate the performance of a novel ultra-sensitive HBsAg assay, the Fujirebio Lumipulse G HBsAg-Quant assay, for screening HBV infection in a large Chinese population.

Methods: A total of 2043 consecutive serum samples for routine pre-surgery testing were tested using the Lumipulse and the Abbott Architect assays in parallel. Samples with inconsistent HBsAg results were subjected to the confirmatory testing using the neutralizing antibodies against HBsAg.

Results: Of the 2043 samples tested, 1844 samples gave negative results and 172 samples yielded positive results using both assays; the remaining 27 samples had inconsistent HBsAg results. Three samples were determined as positive by the Architect assay and 24 samples were determined as positive by the Lumipulse assay. None of the three Architect positive samples were subsequently confirmed as positive. Twenty of the 24 Lumipulse positive samples were confirmed as positive; three samples were confirmed as negative; and the remaining one sample gave an indeterminate result and was subsequently excluded from the specificity calculation for the Lumipulse assay. The specificity was 99.838 % for the Lumipulse assay and 99.840 % for the Architect assay. HBV DNA was measured in the 20 samples with confirmed HBsAg results, among which six samples yielded positive HBV DNA results, ranging from 32 to 600 IU/mL.

Conclusions: The Lumipulse assay is specific for detecting trace amount of serum HBsAg. Approximately 1 % more samples will be reported as positive with the assay. The interpretation of the extremely low-level HBsAg results, however, remains challenging.

P-0329

Antiviral effect of therapy with REP 2139-Ca and nucleos(t)ide analogues against HBV in vivo

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Treatment of human patients with chronic HBV infection with the nucleic acid polymer (NAP) REP 2139 results in the elimination of circulating HBsAg. In this preclinical study we evaluated a novel combination therapy associating REP 2139-Ca with tenofovir disoproxil fumarate (TDF) and entecavir (ETV), in vivo, in the DHBV infection model.

DHBV-carrier ducks were treated for 4 weeks with normal saline (IP), REP 2139-Ca (10 mg/kg IP QD), TDF (15 mg PO QD), REP 2139-Ca + TDF or REP 2139-Ca + TDF + ETV (1 mg PO QD). Serum DHBsAg was monitored by ELISA and DHBV DNA by qPCR. After therapy cessation all animals were followed during additional 8 weeks. Total DHBV DNA and cccDNA were analyzed in autopsy liver samples by qPCR.

On-treatment antiviral responses were more marked when REP 2139 was combined with TDF or TDF and ETV. Sustained virologic responses (SVR) during 2 months off-therapy were only observed in the groups receiving REP 2139 alone or in combination with TDF or TDF and ETV but not in TDF-monotherapy. SVR consisted of stably suppressed serum DHBsAg and DHBV DNA and significant decrease in liver DHBV DNA and cccDNA that were more marked in combination-therapy groups. Importantly, SVR animals had undetectable liver DHBsAg as assessed by immunostaining analysis.

Antiviral performance of REP 2139 was sustained and enhanced by combination with TDF or ETV. Thus an interferon-free regimen of REP 2139 with TDF or ETV could improve antiviral response and shorten treatment regimens in HBV infected patients.

P-0330

Noninvasive diagnosis of hepatic steatosis using fat attenuation parameter and a new algorithm

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Background: Noninvasive approaches for the assessment of hepatic steatosis have been developed as alternatives to liver biopsy. This study compared the diagnostic accuracy of fat attenuation parameter (FAP) measured by transient elastography (FibroTouch) and a new algorithm (fatty index) to assess hepatic steatosis, vs. liver biopsy, in chronic hepatitis B (CHB) patients.

Methods: 254 Consecutive patients with CHB in The Third Affiliated Hospital of Sun Yet-Sen University had simultaneous liver biopsy, biochemical blood tests and FibroTouch (Wuxi HISKY Medical Technologies Co., Ltd. China). A new algorithm was defined, fatty index = $10 * e^{P/(1 + e^P)}$, which was based on four factors (FAP; Body mass index, BMI; High-density lipoprotein, HDL; Apolipoprotein B, APOB) and $P = -2.75 + 0.028 \ln \text{FAP (dB/m)} + 0.409 \ln \text{BMI (Kg/m}^2) - 2.482 \ln \text{HDL (mmol/L)} + 1.979 \ln \text{APOB (g/L)}$. Receiver operating characteristic (ROC) curve analysis was performed for calculating the area under the ROC (AUROC).

Results: There was significant difference in FAP between the two patient groups, CHB and CHB with hepatocyte steatosis ($P < 0.001$). The best cut-off values of FAP for hepatic steatosis $>0, \geq 5, \geq 10, \geq 20$ and ≥ 30 % were 224.1, 230.6, 235.5, 246.9 and 261.1 dB/m, respectively; and AUROCs were 0.833, 0.801, 0.915, 0.917 and 0.972, respectively. The cut-off value of fatty index for diagnosis of hepatic steatosis was 1.5 and AUROC was 0.807 (95 % CI 0.740–0.863, sensitivity = 77.19 %, specificity = 76.52 %, $P < 0.0001$).

Conclusions: Fatty attenuation parameter by transient elastography (FibroTouch) is an accurate, reliable and completely noninvasive approach for the assessment of hepatic steatosis in CHB patients with hepatocyte steatosis.

P-0331

HBV: challenges to cure in the future

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HBV is the second known carcinogen after smoking. 350 millions have Chronic HBV. HBV has ten genotypes (A-J). Genotypes A and D have higher rates of chronicity than genotypes B and C. Genotypes C and D have high rates of cirrhosis and HCC as compared with genotype A and B. HBV may be presented as acute HBV or chronic HBV (Immune tolerant, HBe Ag positive immune active, HBe Ag negative immune active, carrier or occult). Egypt has 90 % genotype D and 90 % HBeAg negative. Vaccination against HBV in infancy is the most effective approach to prevent HBV-related HCC. HBV patients should be screened for HCC every 6 month by US with or without AFP. Also each HBsAg-positive patients should be screened for anti-HDV. Goals of treatment of HBV are long-term suppression of HBV replication and decrease hepatic necroinflammation and fibrosis to prevent progression to cirrhosis and HCC. In the future,

clearance of HBs Ag and cccDNA (covalently closed circular DNA). Candidates eligible for treatment according to are HBe Ag positive or negative immune active phases (PCR more than 2000 and elevated ALT or with moderate to severe liver inflammation), cirrhotic patients with PCR positive, carrier and occult HBV with PCR positive under immune suppression, acute fulminated HBV and decompensated cirrhosis. Candidates ineligible for treatment are immunotolerant, carrier and occult phases. Future strategies to eradicate HBV are targeting the host (immune therapy) and targeting the virus (inhibitors of cccDNA formation and inhibitors of HBV entry into the hepatocyte).

P-0332

In vitro evaluations of anti-hepatitis B activities of 60 medicinal plants extracts

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Although, there are many effective therapeutic drugs available against hepatitis B virus (HBV) infection, they have certain limitations. Therefore, there is an ongoing effort to search for novel potential antiviral herbal products. Though approximately 35 of more than 100 species of Saudi medicinal plants are traditionally used to treat liver disorders, none has been subjected to scientific evaluations for anti-hepatitis B potential. Therefore, in the present study, the anti-HBV activities of total ethanol as well as sequential extracts of 60 candidate medicinal plants were investigated on HBV stable cell line, HepG2.2.15. Of these, 9 plants viz., *G. senegalensis* (dichloromethane extract, IC₅₀ = 10.65); *P. crispa* (ethyl acetate extract, IC₅₀ = 14.45); *C. gardis* (total ethanol extract, IC₅₀ = 31.57); *F. parviflora* (hexane extract, IC₅₀ = 35.44); *C. decidua* (aqueous-extract, IC₅₀ = 66.82); *C. epigeus* (total ethanol extract, IC₅₀ = 71.9); *I. caerulea* (methanol extract, IC₅₀ = 73.21); *A. figarianum* (dichloromethane extract, IC₅₀ = 99.76) and *A. oerfota* (total ethanol extract, IC₅₀ = 101.46) showed marked inhibition of viral HBsAg and HBeAg expressions in a time-dependent manner at non-cytotoxic dose. Further phytochemical analysis of the active extracts showed presence of alkaloids, flavonoids, tannins, and saponins attributed to antiviral efficacies. However, detailed phytochemical study of these extracts is essential to elucidate the active principle(s) responsible for the anti-HBV potential. From the results obtained in this study, it is possible to demonstrate the importance of the application of ethnobotanical information in the search and selection of plants that may provide new opportunities for the treatment of chronic hepatitis B.

P-0333

Evaluation of hepatitis B surface antibody and core antibody in patients with HBsAg-negative

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Aims: We evaluated the positivity rates of hepatitis B core antibody (HBcAb) and hepatitis B surface antibody (HBsAb) in patients without HBsAg.

Methods: In total, 4910 patients with liver disease [chronic hepatitis C infection (CHC) in 2753, non-alcoholic fatty liver disease (NAFLD) in 456, hepatocellular carcinoma (HCC) in 196, liver tumors in 285, alcoholic liver disease (ALD) in 410, autoimmune liver disease in 337, and acute liver injury in 473 patients] who visited our hospital and tested HBsAg-negative by Lumipulse HBsAg-HQ assay (Fujirebio, Japan) in or after 2000 (mean age, 62 years; 2571 men) were evaluated. For measurement of HBsAb and HBcAb, Lumipulse anti-HBs and Lumipulse anti-HBc, respectively, were used.

Results: Of the patients, 2512 (51 %) were HBsAb(-) and HBcAb(-); 195 (4 %) were HBsAb(+) and HBcAb(-); 698 (14 %) were HBsAb(-) and HBcAb (+); and 1505 (31 %) were HBsAb(+) and HBcAb(+). HBsAb and/or HBcAb positivity rates were 12 % in the <30-year age, 17 % from 30 to 39 year age, 29 % from 40 to 49, 48 % from 50 to 59, 56 % from 60 to 69, 59 % from 70 to 79, and 61 % >80. After adjustment for age, the proportions according to diseases were 48 % for ALD, 48 % for CHC, 47 % for autoimmune liver disease, 46 % for HCC, 44 % for liver tumors, 42 % for acute liver injury, and 39 % for NAFLD.

Conclusion: Of the 4910 patients with HBsAg-negative liver disease, 2398 patients (49 %) had a previous hepatitis B virus (HBV) infection. Prevalence of a previous HBV infection was higher in elder patients.

P-0334

HBV cccDNA correlates with histological inflammation activity in chronic hepatitis B infection

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Background: Some researchers reported that intrahepatic HBV cccDNA correlated with liver inflammation grade in cross sectional studies, yet the conclusions were controversial. The aim of this study is to explore in patients infected with HBV with mildly elevated ALT level, whether baseline HBV cccDNA can predict inflammation activation in a prospective cohort study.

Methods: Patients diagnosed of chronic HBV infection with serum ALT level under $2 \times$ ULN were recruited between March 2009 and November 2010 in the center of infectious disease in West China Hospital of Sichuan University. After liver biopsy, serum virological and biochemical markers testing, those with histological grade under G2 accepted a follow up of 4 years, all virological and biochemical markers were retested every 6 months in each patient.

Results: There were 102 patients accomplished the 4 year follow up. Among them 68 individuals were with both baseline ALT under $2 \times$ ULN and liver inflammation grade under G2, 41 of them (60.3 %) developed inflammation activation. HBV cccDNA above 1 copies/cell (OR = 9.43, P = 0.049) independently increased the risk of developing active hepatitis during the 4 year follow up (OR = 9.43, P = 0.049). Baseline HBV cccDNA above 1 copies/cell can predict inflammation activation (AUC = 0.663, P = 0.034) with stronger efficiency compared with serum HBsAg (AUC = 0.634, P = 0.063) and HBV DNA (AUC = 0.600, P = 0.166).

Conclusions: Higher baseline intrahepatic HBV cccDNA level may increase the risk of developing inflammation activation in near future, indicating HBV cccDNA may be applied as an intrahepatic virological marker in selecting those who requiring fortified outpatient management.

P-0335

The association between quantitative hepatitis B core antibody level and HBV seromarkers

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We investigated the associations of qAnti-HBc with HBV markers in our community-based REVEAL-HBV cohort. qAnti-HBc levels were examined by a new double-antigen sandwich immunoassay in 3050 participants seropositive for hepatitis B surface antigen (HBsAg) at study entry. qAnti-HBc levels were log-transformed. Linear regression models were used for studying the association between qAnti-HBc levels and HBV seromarkers. In the multivariate linear regression analysis in HBeAg-seronegative participants, elevated HBV DNA, HBsAg, and ALT levels were significantly associated with increasing qAnti-HBc (beta-coefficient [95 % CI] = 0.54 [0.44–0.65] for HBV DNA \geq 10⁶ copies/mL compared to <10³ copies/mL, 0.19 [0.13–0.26] for HBsAg levels 102–103 IU/mL compared to <2 IU/mL, and 0.13 [0.01–0.24] for ALT levels \geq 45 IU/mL compared to <45 IU/mL). Elevated HBV DNA and ALT were also associated with higher qAnti-HBc in HBeAg-seropositive participants (beta-coefficient [95 % CI] = 0.70 [0.11–1.29] for HBV DNA \geq 10⁶ copies/mL compared to <10³ copies/mL and 0.30 [0.17–0.43] for ALT levels \geq 45 IU/mL). Among participants with HBV DNA \geq 10⁶ copies/mL, those with genotype B or B and C had higher qAnti-HBc than those infected with genotype C (mean log₁₀[anti-HBc] level, 4.14 vs. 3.78; beta-coefficient [95 % CI] = –0.30 [–0.48 to –0.12]). Participants with elevated ALT levels still had higher qAnti-HBc (beta-coefficient [95 % CI] = 0.65 [0.43–0.87] for ALT levels \geq 45 IU/mL compared to <45 IU/mL). Several viral factors are associated with qAnti-HBc and should be considered when evaluating the effect of qAnti-HBc on clinical outcomes.

P-0336

Neopterin as a marker for immune status in patients with chronic hepatitis B

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Objective: Neopterin, produced by macrophages, serves as a marker of cellular immune system activation. The aim of the study was to evaluate a correlation between the serum neopterin level and natural course of chronic hepatitis B infection.

Method: A total of 60 patients were divided into three groups; 9 immune tolerance (HBVDNA >2000 IU/mL, ALT <40 U/L, and HBeAg positive), 13 immune clearance (HBVDNA >2000 IU/mL, ALT \geq 40 U/L, and HBeAg positive), and 38 inactive carrier phase (HBVDNA <2000 IU/mL, normal ALT, and HBeAg negative). Serum neopterin levels were measured by the method of competitive enzyme-linked immunosorbent assay. Kruskal–Wallis was used for statistical analysis.

Results: HBV DNA, ALT, and neopterin (nm/L) levels of the patients according to immunologic phases as follows: 1.7 \times 10⁸ (1.3 \times 10⁷ to 6.7 \times 10⁸), 28 (20–44), and 7.43 (3.11–13.85) in immune tolerance phase, 1.7 \times 10⁸ (1.4 \times 10⁵ to 6.7 \times 10⁸), 84 (59–193), and 15.47 (3.01–27.47) in immune clearance phase, and 1.6 \times 10³ (20 to 1 \times 10⁵), 26 (10–63), and 6.75 (2.44–15.51) in inactive carrier status, respectively. Serum neopterin level was found higher in immune clearance phase than the others (p < 0.001). No difference was found between the immune tolerance and inactive carrier phases. High neopterin levels were correlated with high ALT levels. HBV DNA levels were not associated with the neopterin levels.

Conclusions: Measurement of the serum neopterin level will provide additional immunologic information about the status of CHB. Elevated serum neopterin level may be as a useful predictor of the immune clearance phase of the infection. However, it may not indicate viral replication status.

P-0337

Red cell distribution width to albumin ratio, a novel index of the severity of chronic HBV infection

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Background: Simple and inexpensive non-invasive fibrosis tests are highly needed to assess the severity of HBV infection. We identified a new simple laboratory index, the red cell distribution width (RDW)-to-albumin ratio (RAR), to assess the severity of chronic HBV infection and compare it with well-known noninvasive predicting fibrosis scores including aspartate aminotransferase-to-platelet ratio index (APRI), the fibrosis-4 score (FIB-4), neutrophil-to-lymphocyte ratio (NLR), RDW-to-platelet ratio (RPR), gamma-glutamyl transpeptidase to platelet ratio (GPR) and alanine aminotransferase ratio (AAR). Methods: Ninety-eight CHB patients and 112 patients with HBV-related liver cirrhosis were enrolled. One hundred and ninety-seven healthy individuals were included as control. AUROC curve was used to calculate predicting performance of RAR and compared with other noninvasive fibrosis indexes.

Results: The RAR was significantly higher in patients with HBV-related liver cirrhosis compared to CHB patients and healthy controls (all P < 0.001). RAR was positively correlated with Child-Pugh (r = 0.655, P < 0.001) and MELD scores (r = 0.29, P = 0.002). The ROC curve yielded a sensitivity of 84.82 % and a specificity of 85.71 % at a threshold value of 0.365 for RAR in prediction of HBV-related liver cirrhosis. For predicting cirrhosis, area under the ROC curve of RAR (0.931) was significantly better than that of APRI (0.675, P < 0.0001), FIB-4 (0.859, P = 0.002), NLR (0.662, P < 0.0001), GPR (0.729, P < 0.0001) and AAR (0.817, P = 0.0005).

Conclusion: RAR can be used as a novel non-invasive marker to assess the severity of HBV-related liver diseases. The RAR is a more accurate routine laboratory marker than APRI, FIB-4, NLR, RPR, GPR and AAR to predict HBV-related liver cirrhosis.

P-0338

Selected cytokines predicting liver histology in CHB patients with normal to mildly elevated ALT

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To date there have been no reliable biomarkers for assessing histological liver damage in chronic hepatitis B(CHB) patients with normal or mildly elevated alanine aminotransferase (ALT). The aim of the present study was designed to assess the utility of circulating cytokines in diagnosing liver inflammation and fibrosis in CHB patients with ALT less than 2 times the upper limit of normal range (ULN). All patients were treatment naive and underwent liver biopsy and staging by Ishak system. Twenty one cytokines were detected by luminex screening system. The 31.8 % and 29.1 % of 151 patients with ALT $<2 \times$ ULN had at least moderate inflammation and significant fibrosis, respectively. Multivariate analysis demonstrated that CXCL-11 was independently associated with at least moderate inflammation, and TGF- α and IL-2R independently correlated with significant fibrosis in patients with ALT $<2 \times$ ULN. Based on certain cytokines and clinical parameters, an inflammation-index and fib-index were developed, which showed areas under the receiver operating characteristics curve (AUROC) of 0.75 (95 % CI 0.66–0.84) for at least moderate inflammation and 0.82 (95 % CI 0.75–0.90) for significant fibrosis, correspondingly. Compared to existing scores, fib-index was significantly superior to aspartate aminotransferase (AST) to platelet ratio index (APRI) and FIB-4 score for significant fibrosis. In conclusion, CXCL-11 was independently associated with at least moderate inflammation, whereas IL-2R and TGF- α were independent indicators of significant fibrosis in patients with normal or mildly elevated ALT. An IL-2R and TGF- α based score (fib-index) was superior to APRI and FIB-4 for the diagnosis of significant fibrosis in patients with normal or mildly elevated ALT.

P-0339

HBV reduces IRF3 gene expression despite increased expression of cellular cytosolic DNA sensor cGAS

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Background: cGAMP synthase (cGAS) is a cytosolic DNA sensor. Interaction between cGAS and Cytosolic DNA activates the cGAS to synthesize cyclic GMP-AMP (cGAMP) that triggers IRF3 pathway by binding to a downstream adaptor protein called STING. Hepatitis B virus (HBV) during its DNA replication gains access to the cytosol, that might elicits a potent antiviral interferon response through cGAS. Whether viral DNA in an infected cell, get sensed by the above mechanism or interrupted by the virus at certain stage is not known.

Objective: The present study was undertaken to assess the expression of cGAS, STING and IRF3 mRNAs in HBV infected PBMC obtained from patients.

Methods: Sixty HBsAg positive patients were evaluated for serum HBeAg, antiHBe and ALT status by ELISA and biochemical procedures respectively. Quantification of HBVDNA levels were estimated by real-time PCR assay. cGAS, STING and IRF3 mRNA expression was assessed by quantitative reverse transcriptase PCR assay. Thirty voluntary blood donors served as controls.

Results: Expression of cGAS and STING mRNA levels were significantly higher ($P < 0.0001$) in patients (6.9 ± 0.8 , 6.4 ± 1.8) in comparison to controls (5.8 ± 0.5 , 4.2 ± 2.9) respectively. Whereas IRF3 mRNA level was significantly lower ($P < 0.0001$) in patients (6.4 ± 1.3) in comparison to controls (8.15 ± 0.7).

Conclusion: Despite the up regulation of host cGAS and STING expression in HBV infected cell, significant suppression of IRF3 expression was also observed, which indicates HBV induced derangement of downstream signaling pathway of cGAS-STING-IRF3 axis inhibiting production of type 1 interferon. This can be considered as a part of survival strategy of HBV for its persistence.

P-0340

Characteristics of HBV full-length sequences in 21 persons among 509 residents in Binh Thuan, Vietnam

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Background: According to WHO, Vietnam is one of the countries with the highest mortality from liver cancer and had a high age-standardized mortality for liver cancer (23.7 per 100,000) in 2012. Approximately 85 % of Vietnamese with HCC had evidence of HBV infections. We investigated characteristic of HBV infections, and full-length sequences of HBV-DNA among residents in Binh Thuan, Vietnam.

Methods: We conducted a serologic testing for HBV infections, and family-tree survey for HBV-DNA sequencing with written IC from 510 participants in Vietnam. HBsAg was quantified using a reversed passive hemagglutination assay, while HBsAb and HBeAb were detected using a chemiluminescence immunoassay. Subsequently, HBsAg positive participants and their family members were invited to a family tree survey. In the family-tree survey, HBV-DNA was detected by real-time PCR, and the obtained full-length sequences of HBV were determined by direct sequencing and phylogenetic analysis.

Results: In total, 509 participants (40.8 ± 1.1 years) were included in the serologic testing. Prevalence of HBsAg, HBsAb, and HBeAb were 15.3, 60.3, and 71.7 %, respectively. In the family-tree survey, 47 sequences of DNA polymerase and 21 full-length sequences were obtained. All family members were genotype B4 and most sequences from same family were similar to each other but some sequences of intra-familial member were more similar to sequences from extra-familial participants than intra-familial members.

Conclusion: According to the result of full-length sequencing, we could not find obvious evidence of perinatal HBV infections. Horizontal infections might have occurred not only among family members but also among residents.

P-0341

Biological characteristics and impacts of truncated HBsAg mutant expressed by HBV rtA181T mutationLingyun Zhou¹

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Background: Mutation in the hepatitis B virus (HBV) P gene can cause the overlapped S gene alteration. This study aims to investigate the biological characteristics of the truncation mutant strain located on the S gene caused by P gene mutations.

Methods: This study utilized cell transfection and hydrodynamic injection to construct mutant HBV replication cell models and mice models to explore the biological characteristics of the truncation (rtA181T/sW172*) mutation; used PCR to obtain the S genes, constructed the eukaryotic expression plasmids and established the stable expression cell lines to find out the differences about cell growth activity.

Results: In HepG2 cell supernatant, HBsAg of rtA181T/sW172* was weak positive and the HBV DNA load decreased compared to wildtype. HBsAg of rtA181T/sW172* in mice serum could only be detected with weak positive characteristics on day 3 after injection. In mice liver: most of the HBV DNA replication intermediates levels of rtA181T/sW172* mutation were higher than wildtype. The HBsAg and HBcAg of rtA181T/sW172* stained hepatocytes were abundant from day 3 to day 15. The relative luciferase activity of CpLUC in pcDNA-HBs(sW172*) group increased. The expression level of truncated HBsAg was significantly higher, the LO2-pcDNAHBs(sW172*) cell line proliferated more rapidly and the proportion of S phase was higher than other cell lines.

Conclusions: HBV rtA181T/sW172* mutation has a dominant secretion defect of HBsAg and enhances HBV replication intracellular. The truncated HBsAg could transactivate HBV Cp. The truncated HBsAg might promote cell proliferation and the mechanism might be related to the activation of G1/S transition.

P-0342

Comparison of HBV variants by deep sequencing with PCR and without PCRYujiao Liang¹, Yoshihiko Yano^{1,2}, Laura Navika Yamani¹, Widya Wasityastuti¹, Rina Okada², Toshihito Tanahashi³, Yoshiki Murakami⁴, Takeshi Azuma², Yoshitake Hayashi¹

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Background: Recent technological advancement of next-generation sequencer made it easy to analysis viral quasispecies of HBV genome. In general, PCR product is used as a template, but the result of minor variants can be affected by multiple amplification.

Method: Viral HBV-DNA was collected from 6 HBV infected patients and extracted. Each sample was divided into two. One was treated by nuclease and another was used for PCR. The two kinds of products were sequenced using Illumina MiSeq sequencer. To

evaluate the methodological difference, the population of minor variants of deep sequencing using nuclease-treated DNA product and PCR product were compared. The variants were defined based on the percentage of the viral population affected: major, >20 % of the total population; intermediate, 5 % to <20 %; and minor, 1 % to <5 %.

Result: The detection rate of minor population by PCR products was significantly higher than that by nuclease treated DNA products (3.72 vs. 2.34 %, $p = 0.01$) The difference was significant in C region (2.79 vs. 1.36 %, $p < 0.01$), but less significant in S region. Most of minor amino acid variant were detected by both of PCR and DNA method.

Conclusion: Although PCR is a necessary step for the detection of small amount of HBV-DNA, it should be careful the detection of minor variants using PCR products. The amplification efficacy is different depend on the genomic region, and further experience will be is necessary.

Keywords: HBV variant, deep sequencing, minor population, PCR

P-0343

Characterization of HBV whole genome by ultra deep sequencing during acute infection of G145R mutantYuan Xue^{1,2}, Ming-jie Wang^{1,2}, Zhi-tao Yang^{1,2}, De-min Yu^{1,2}, Yue Han^{1,2}, Dao Huang^{1,2}, Dong-hua Zhang¹, Xin-Xin Zhang^{1,2}

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Background: Amino acid (aa) substitution from glycine to arginine at aa 145 (G145R) in the major hydrophilic region of hepatitis B virus (HBV) surface antigen (HBsAg), can cause reduced binding of anti-HBs, resulting in HBsAg/anti-HBs coexistence and immune escape.

Methods: Serological changes and whole genome quasispecies characteristics during acute infection of G145R mutant HBV were described in a patient with prior anti-HBs positive.

Results: Alanine aminotransferase peaked at the 16th week, while HBsAg and HBV e antigen (HBeAg) declined rapidly. HBsAg clearance and HBeAg/anti-HBe seroconversion were achieved at the 36th week. Sequence analysis with Illumina Miseq showed that complexity and genetic distances of s and rt regions were much higher at the 8th week compared to baseline and the 4th week. Moreover, frequency of L110I, T113S and I126T variants increased at the 8th week, while that of G145R, K160R, L173P and Q181R variants decreased.

Conclusions: Ultra-deep sequencing provides insights into the quasispecies characteristics of G145R mutant HBV.

Keywords: Hepatitis B virus, quasispecies, mutation, ultra-deep sequencing

P-0344

Quasispecies variants of surface HBV detected by ultra-deep sequencing in Indonesian patientsLaura Navika Yamani^{1,2}, Yoshihiko Yano^{1,3}, Takako Utsumi^{1,2}, Juniastuti Juniastuti², Hadi Wandono⁵, Doddy Widjanarko⁶, Ari Triantanoe⁶, Widya Wasityastuti^{1,4}, Yujiao Liang¹, Rina Okada³, Toshihito Tanahashi⁷, Yoshiki Murakami⁸, Takeshi Azuma³, Soetjipto Soetjipto², Maria Inge Lusida², Yoshitake Hayashi¹

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The high mutation rate of hepatitis B virus (HBV) replication generates genetic diversity, referred to as a viral quasispecies. Quasispecies of HBV surface gene might have an influence on antigenicity and immunogenicity of the virus, affecting the clinical outcome of the infected patient. The analysis using ultra-deep sequencing might be possible to quantify the true proportion of quasispecies population. In our investigation, the exact number of variants population in surface gene were examined by ultra-deep sequencing among 11 chronic hepatitis (CH) (mean age 52.55 ± 9.417 years) and 19 advanced liver disease (ALD) (mean age 52.28 ± 11.671 years) of Indonesian patients. The average HBsAg titer in patients with ALD (21.506 ± 81.294 IU/ml) was lower than that in CH (421.520 ± 737.955 IU/ml). Quasispecies variants detected from ultra-deep sequencing were clustered based on the proportion in the total population. The frequency of surface variants in ≥ 1 and ≥ 5 % of total population were significantly higher in patient with ALD than CH. Whereas, only the frequency of MHR variants with population ≥ 5 % was higher in ADL than CH patients. Mutations in B, Th and CTL cell epitopes were found more frequently in ADL patients. These mutants may cause that the virus evades humoral and cellular immunity, eventually cause the viral persistence. It was concluded that a greater number of surface variations was related to disease severity and reduced likelihood of HBsAg titer. This is the first study to use ultra-deep sequencing for the detection of surface variants of HBV quasispecies in Indonesian patients.

Keyword: Surface variations, ultra-deep sequencing, Indonesian.

P-0345

Spectrum of HBV DNA drug resistance mutations in Korea: is heteroresistance of HBV emerging?

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The emergence of drug-resistant hepatitis B virus (HBV) with mutations induced by nucleoside/nucleotide analogue treatments of chronic hepatitis B (CHB) is the major concern to achieve successful treatment and management of CHB. Among various methods to detect HBV mutations including reverse hybridization and ultra-deep pyrosequencing, restriction fragment mass polymorphism analysis using mass spectrometry is sensitive and suggested for fast and multiplex nature of the assay. Herein, we report the spectrum of HBV mutations in Korea. A total of 382 serum samples from 358 patients during 20 months (from January 2014 to August 2015) were tested. Multiplex PCR was performed with primers designed to interrogate rt180, rt181, rt184, rt202, rt204, rt236 and rt250. The PCR products were digested with restriction enzymes and the resulting fragments were analyzed by mass spectrometry. Among 261 samples in which HBV DNA were detected, 29.5 % were negative for mutations at all 7

loci in the test. While lamivudine resistance associated mutation was predominant (40.2 %), entecavir resistance mutation with prerequisite lamivudine resistance mutation and adefovir resistance mutation comprised 16.5 % and 10.0 %, respectively. L180M, A181V, and S202G were the most common mutations for lamivudine, adefovir, and entecavir resistance, respectively. Interestingly, 30.6 % of mutant HBV DNA showed results for mixed population of wild type and mutant strains, suggesting heteroresistance for antiviral agents. In conclusion, we report the current spectrum of drug resistant HBV DNA mutations in Korea. Large proportion of heteroresistance should be considered for further treatment of drug-resistant HBV.

P-0346

Incidence of spontaneous resistance mutation in untreated chronic hepatitis B patients in China

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Background: Long-term nucleos(t)ide analogues therapy can lead to hepatitis B virus (HBV) resistance, while spontaneous resistance mutations in treatment-naïve chronic hepatitis B (CHB) patients have been reported. Since an increasing studies focused on HBV mutation rate in untreated Chinese CHB patients have aroused controversy, we conducted this meta-analysis to appraise the pooled incidence of natural mutations in China.

Methods: We searched PUBMED, EMBASE, CBM and CNKI till October 31st, 2014. Two investigators independently selected cross-sectional or case-control studies reporting incidence of natural resistance mutations in untreated CHB patients in China. Pooled incidence was performed in fixed or random effects models using R software, and heterogeneity was assessed.

Results: A total of 72 studies were included involving 10,256 naïve Chinese CHB patients. The summarized incidence of natural mutations in China was 6.87 % (95 % CI 5.71–8.03 %). Southern China had a little higher pooled mutation rate than Northern China. Primary mutation rtM204 V/I had the highest incidence of 6.42 % (95 % CI 5.35–7.48 %), and others seldom spontaneously occurred. From 2001 to 2014, the highest annual spontaneous mutation rate reached up to 12.78 % and the accumulated rate floated between 6.00 and 8.00 % mostly. In subgroup analysis, genotype C HBV infection, male and hepatitis B antigen negative patients had a slightly higher natural mutation rate.

Conclusions: The resistance mutations occurred frequently in untreated CHB patients in China. The lamivudine resistance had the highest natural prevalence rate, while other nucleos(t)ide analogues showed rarely spontaneous resistance. Detecting the spontaneous resistance mutations will benefit the clinical management of CHB patients.

P-0347

HBV DNA levels on HBe seroconversion could predict clinical course in chronic hepatitis B

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Background and Aim: Hepatitis B e antigen (HBeAg) seroconversion (SC) has been regarded as one therapeutic goals for HBeAg-positive chronic hepatitis B (CHB), so far. However, a lot of CHB patients develop cirrhosis and/or hepatocellular carcinoma, despite of HBeAg SC status. There were little reports described whether HBV markers around HBeAg SC could be related with disease deterioration or not. The aim of this study was to evaluate relationship between HBV status on HBeAg SC and subsequent clinical outcome of CHB.

Methods: Retrospective investigation was performed for 136 patients with HBeAg-positive CHB followed between April 1997 and October 2013. The patients treated with antivirals around HBeAg SC were excluded. Cohort was completed when antivirals were initiated.

Results: A total of 16 patients (10 males, 35 median y-o) were enrolled out of 37 who showed HBeAg SC. HBeAg SC was achieved after 26 (1–75) observation months, and, 12 out of them had the past antiviral therapies prior to SC, interferon or interferon + lamivudine. Because of sustaining active hepatitis, 8 of 16 enrolled patients were given nucleos(t)ide analogues posterior to the HBeAg SC. In these 8 patients with disease deterioration, HBV DNA levels were higher at the end of observation (6.9 logCp/ml) and even on the timepoint of HBeAg SC (5.2 logCp/ml) compared with those in another 8 patients without deterioration (2.9 logCp/ml, $p = 0.00001$, 2.7 logCp/ml, $p = 0.009$), respectively.

Conclusions: HBV DNA levels on HBeAg SC may predict clinical outcome of hepatitis posterior to the HBeAg SC.

P-0348

Hepatitis B surface antigen (HBsAg) levels and hepatitisBeAntigen (HBeAg) seroconversion status

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Background/Aims: HBsAg seroconversion is considered the gold standard for cure. We explore the association between HBsAg level (iu/ml) and HBeAg seroconversion (HepatitisBeAntigen (HBeAg) negative /Hepatitis B eAntibody(HBeAb)positive) among the different ages. Favourable level for HBsAg is <1500 iu/ml while good prognosis is <150 iu/ml. All had vertical transmission.

Methods: Fifty patients from the ages of 20–75 years old with chronic hepatitis B were followed up from 2012–2014, Hospital Tengku Ampuan Afzan, East Coast of Malaysia. Bloods were sampled simultaneously for HBsAg levels and HBeAg/HBeAb status.

Results: For 20–30 years old, 100 %, $n = 9$, had HBsAg >1500 iu/ml (33 %, $n = 3$, >20,000 iu/ml). For 31–40, 56 %, $n = 9$, >1500 iu/ml (13 %, $n = 2$, >20,000 iu/ml and 25 %, $n = 4$, <150 iu/ml). For 41–50, 27 %, $n = 4$, >1500 iu/ml (6 %, $n = 1$, >20,000 iu/ml and 20 %, $n = 3$, <150 iu/ml). For 51–75, 30 %, $n = 3$, >1500 iu/ml (40 %, $n = 4$, <150 iu/ml. HBeAg seroconversion rate was, 20–30 years old (57 %, $n = 4$), 31–40 (75 %, $n = 12$), 41–50 (87 %, $n = 13$) and 51–75 (90 %, $n = 9$) respectively. Rate of seroconversion was 91 % for <150 iu/ml, 86 % between 150–1500 iu/ml, 68 % between 1500–20,000 iu/ml and 50 % >20,000 iu/ml.

Conclusion: Favourable HBsAg levels (<1500 iu/ml) were more common among older age groups (30 and above). Higher levels (>20,000 iu/ml) were more common among younger age group (<30). HBeAg seroconversion was more common among older age groups (30 and above). HBeAg seroconversion rate was closely associated with low levels of HBsAg and this trend was associated

with advancing age regardless of status of treatment. HBsAg (>20,000 iu/ml) in older age groups (>30) were more common amongst seroconverted patients compared to the younger age group (<30), suggestive of hepatitis B e antigen-negative chronic hepatitis B.

P-0349

Hepatitis B core antibody levels can predict HBeAg seroclearance in chronic hepatitis B patients

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Background: Recent preliminary studies have shown that quantitative Anti-HBc may play an important role in the natural history of chronic hepatitis B. This study examines the ability of Anti-HBc levels to predict HBeAg seroclearance in a community-based cohort of untreated individuals.

Methods: A total of 373 HBeAg-seropositive patients were included in this cohort. HBeAg seroclearance was determined by long-term follow-up, and baseline Anti-HBc levels were quantified by a double-sandwich immunoassay. Anti-HBc levels were log-transformed for analysis, and rate ratios (95 % confidence intervals) were determined by Cox Proportional Hazards models.

Results: Among 373 patients, 172 cases of HBeAg seroclearance occurred over 2576.2 person-years of follow-up, for an overall incidence rate of 667.7 per 10,000 person-years. Increasing levels of Anti-HBc were associated with increasing rates of HBeAg seroclearance. In multivariate analyses, higher Anti-HBc levels were associated with increasing rates of HBeAg seroclearance, with adjusted (95 % CI) rate ratios of 2.55 (1.18–5.53), 3.92 (1.96–7.86), 4.28 (2.24–8.19), and 5.65 (2.85–11.18) for Anti-HBc levels of 3–3.5, 3.5–4, 4–4.5, and >4.5 log₁₀ IU/mL, compared to those with levels <3 log₁₀ IU/mL. In sensitivity analyses among 311 patients also adjusting for viral genotype and the precore G1896A mutation, multivariate rate ratios (95 % CI) of HBeAg seroclearance were still significant, ranging from 3.53 (1.21–10.27) to 8.44 (3.26–21.85) for Anti-HBc levels of 3–3.5 and >4.5 log₁₀ IU/mL, respectively.

Conclusion: This study shows that increasing Anti-HBc levels are associated with higher rates of HBeAg seroclearance, and supports the importance of monitoring Anti-HBc levels during management of chronic hepatitis B patients.

P-0350

The evaluation of the factors affecting seroconversions in HBeAg+ chronic hepatitis B patients

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HBeAg seroconversion mark a transition from the immune-active phase of disease to the inactive carrier state. Spontaneous or treatment-induced HBeAg seroconversion is associated with lower rates of disease progression to cirrhosis and hepatocellular carcinoma, a potential of hepatitis B surface antigen seroconversion, and improved survival rates. This study was designed to assess the HBeAg seroconversion rates and the factors affecting seroconversion in our patients with HBeAg(+) chronic hepatitis B (CHB). In this study, charts of 69 adult patients with HBeAg(+)CHB were reviewed retrospectively between 2003–2013). Patients' demographic data, liver histopathology, biochemical, virological data were recorded. The patients who accomplished HBeAg seroconversion were compared with those who did not, for sorting out factors predictive of HBeAg seroconversion. The mean age and follow up duration were 35.9 and 3.6±2 years respectively. Patients were evaluated in 4 groups. Patients in group immunotolerant (n = 11, 15.9 %) were followed by without treatment. Patients in group treatment were divided into 3 subgroups; the patients treated with interferon and subsequent nucleoside/nucleotide analogues (NAs) (group 1, n = 7, 10.1 %), the patients treated with different NAs (group 2, n = 11, 15.9 %) and the patients treated with same NA, either lamivudine, adefovir, entecavir, or tenofovir (group 3, n = 40, 58 %). Of the patients, a total of 22 achieved HBeAg seroclearance (32 %). The comparison of the seroconversion rates means, there was no significant differences among the groups (group immunotolerant and treatment groups) (p > 0.05). Also different NA treatments in group 3 did not have any statistically significant difference regarding HBeAg seroconversion (p > 0.05).

Conclusion: The levels of under 2000 IU/ml in HBVDNA and decrease in HBVDNA of more than 2 log₁₀ IU/ml at 6 months of treatment are useful for predicting HBeAg seroconversion.

P-0351

HBeAg is a predictor of HBeAg seroconversion in CHB patients treated with pegylated interferon

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Background: To evaluate the usefulness of serum hepatitis B virus core-related antigens (HBcrAg) for predicting HBeAg seroconversion in HBeAg-positive chronic hepatitis B patients treated with conventional interferon (IFN) alfa-2b or PegIFN.

Methods: Fifty-eight patients were enrolled; 29 for the training group and 29 for the validating group. HBcrAg was carried out at baseline, week 12, end of treatment, follow-up week 12 and follow-up 24 week, respectively, for two groups. Sixteen patients in the training group were followed up for 8.8 years.

Results: HBcrAg level gradually declined through treatment in HBeAg seroconversion patients of the training group. HBcrAg less than 19,565 kU/mL at week 24 showed strong potential of HBeAg seroconversion at the end of follow-up. HBcrAg less than 34,225 kU/mL at follow-up week 12 can predict HBeAg seroconversion at the end of follow-up. More than or equal to 0.565 log₁₀ kU/ml decrease in HBcrAg level from baseline at 24 week of therapy showed strong potential of HBeAg seroconversion at the end of follow-up. Cutoff of

HBcrAg at end of treatment and follow-up week 12 from the training group had similar PPV and NPV in the validating group. The patients with HBeAg seroconversion at the end of 24-week follow-up remained seroconversion during long-term follow-up (LTFU).

Conclusions: Effective antiviral treatment can decrease HBcrAg levels in serum. HBcrAg at week 24 and at follow-up week 12 can predict HBeAg seroconversion at follow-up week 24. HBeAg seroconversion after treatment with PegIFN alfa-2b is sustained during LTFU.

P-0352

Baseline HBeAg and CH50 predicts early HBeAg seroconversion in chronic hepatitis B patients

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Background: Many researchers focus on predictors for HBeAg seroconversion after antiviral treatment. However, Data regarding the predictors for early HBeAg seroconversion are limited during antiviral treatment in chronic hepatitis B (CHB) patients. The aim of this study was to find a useful predictor for early HBeAg seroconversion at week 24.

Methods: We conducted a prospective study enrolled 84 CHB patients treated with entecavir. Logistical regression and area under receiver-operator curve (AUC) were used to determine the diagnostic accuracy of simple tests for early HBeAg seroconversion.

Results: At the end of this study, 14 patients (16.7 %) achieved HBeAg seroconversion at week 24. No direct correlation was found between HBVDNA and early HBeAg seroconversion; however, baseline HBeAg and CH50 was associated early HBeAg seroconversion in all patients (r = -0.574, -0.469 respectively). By logistical regression analysis, baseline HBeAg and CH50 were identified as predictors for early HBeAg seroconversion. Thus, a logistic model based on baseline HBeAg and CH50 was selected for the subsequent analysis. The areas under ROC curves (AUC) were 0.911 for early HBeAg seroconversion with a sensitivity of 91.7 % and a specificity of 85.7 %.

Conclusion: A logistic model based on baseline hepatitis B e antigen and CH50 strongly predicts early HBeAg seroconversion at week 24 in HBeAg positive chronic hepatitis B patients treated with entecavir.

P-0353

SLC10A1 variant is associated liver fibrosis in patients with chronic hepatitis B

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Background and aims: Sodium-taurocholate cotransporting polypeptide (NTCP) encoded by SLC10A1 is a cellular receptor for

hepatitis B virus (HBV) in human infection. The aim of this study was to investigate the role of SLC10A1 rs2296651 variants on liver fibrosis progression in patients with chronic hepatitis B (CHB).

Methods: This was a prospective cohort study. Inactive CHB patients with inactive disease, which was defined as negative hepatitis B e antigen (HBeAg), HBV DNA <20,000 IU/ml, normal alanine aminotransferase (ALT) and without advanced fibrosis at baseline underwent liver stiffness measurement (LSM) by transient elastography. 342 patients who remained treatment naive at the second transient elastography examination at an interval of 44 ± 7 months were genotyped for SLC10A1 rs2296651 (c.800C>T) variant.

Results: The mean age was 48 ± 11 years, 51 % were males, ALT was 28 ± 11 IU/l, HBV DNA was 2.7 ± 1.0 log IU/ml, and LSM was 5.4 ± 1.5 kPa. The prevalence of wild-type homozygotes (CC) and heterozygotes (CT) was 79.8 and 20.2 % respectively. Patients of heterozygous SLC10A1 genotype were more likely to have insignificant fibrosis defined by LSM at baseline, when compared to those of wild-type homozygotes (89.9 vs. 62.3 %, $P < 0.001$). The heterozygotes tended to have lower risk of liver fibrosis progression at an interval of 44 ± 7 months (1.4 vs. 3.7 %; $P = 0.352$). Patients of heterozygous SLC10A1 genotype were more likely to be infected by genotype B HBV, when compared to those of wild-type homozygotes (72.7 vs. 51.9 %, $P = 0.028$).

Conclusions: SLC10A1 rs2296651 CT genotype is associated with milder liver fibrosis and HBV genotype B infection.

P-0354

SNPs are associated with pathogenesis of chronic HBV infection

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Purpose: GWAS revealed that single nucleotide polymorphism (SNP) acting in favor of clearance of HBs antigen in the early stage of the infection are A allele of rs3077 (HLA-DP), and A allele of rs9277535 (HLA-DP), but there are a few reports on the effects of the SNPs in the patients with chronic HBV infection. We examined the effects of the SNPs on the pathogenesis of HBV in the patients with chronic HBV infection and HBe antigen negative.

Methods: The SNPs of HLA-DP were determined in 231 patients with chronic HBV infection and HBe antigen negative. The patients were divided into GG group and AA/AG group according to the SNP of rs3077 and rs9277535, respectively. Quantity of HBs antigen, and core-related antigen, and presence of the nucleic acid analog treatment were compared between the groups.

Results: GG group and AA/AG group of rs3077 and rs9277535 did not show a significant difference in quantity of HBs antigen, and core related antigen. The patients with treatment of nucleic acid analog were significantly fewer in AA/AG group (32 %) than in GG group (44 %) in rs3077 ($p = 0.0499$). Those with treatment of nucleic acid analog were also significantly fewer in AA/AG group (32 %) than in GG group (45 %) in rs9277535 ($p = 0.0414$).

Conclusions: In HBe antigen-negative patients, AA/AG group were receiving nucleic acid treatment significantly less frequently than GG group both in rs3077 and in rs9277535. Thus A allele of rs3077 and

rs9277535 was thought to be associated with less active disease in chronic HBV infection.

P-0355

The association between SNPs on ADH1B and ALDH2 and HBV related HCC is mediated by alcohol drinking

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Background and Aims: The single nucleotide polymorphisms (SNPs) rs1229984 (ADH1B) and rs671 (ALDH2) play crucial roles in regulating alcohol metabolism. This study examines the interaction of these two polymorphisms with alcohol drinking and risk of HCC among chronic hepatitis B patients.

Methods: A total of 3824 individuals with chronic hepatitis B were enrolled in this study. Two SNPs, rs1229984 (ADH1B) and rs671 (ALDH2) were genotyped as a part of a genome-wide association study using the Affymetrix Axiom Genome-wide CHB1 Array. Direct (DE) and indirect (IE) effects of the two SNPs on HCC risk, as mediated through alcohol drinking, were examined using mediation analyses.

Results: A total of 602 cases of HCC were included. The frequencies of the rs1229984 T allele and rs671 A allele were 72.9 and 28.8 %, respectively. Individuals who carried both SNPs were significantly less likely to become habitual alcohol drinkers, with an odds ratio (OR) and [95 % confidence interval (CI)] of 0.22 [0.12–0.39]. Rs1229984 (ADH1B) and rs671 (ALDH2) were not independently associated with HCC in multivariate analyses. However, mediation analyses showed that the rs1229984 T allele, rs671 A allele, and the two SNPs combined were significantly indirectly associated with decreased HCC risk, mediated through habitual alcohol drinking, with an OR (95 % CI) of 0.87 (0.79–0.96), 0.70 (0.61–0.82), and 0.73 (0.58–0.88), respectively, with no significant SNP by alcohol interaction.

Conclusions: Although single nucleotide polymorphisms on ADH1B and ALDH2 did not directly affect HCC risk, they were protective against HCC through their protective effect against habitual alcohol drinking.

P-0356

Association between HBV infection and HLA gene rs3077 and rs9277535 in the Turkish population

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Background: Hepatitis B virus (HBV) affects approximately 360 million people worldwide. 15 % of chronic carriers develop liver cirrhosis (LC), liver failure and hepatocellular carcinoma (HCC). Chronic HBV infection or HBV clearance is influenced by both viral and host factors. In genome-wide association studies (GWAS), the human leukocyte antigen (HLA) gene polymorphisms rs3077 and rs9277535 were identified to be associated with chronic hepatitis B. HLA genes have been linked to immune response to infectious agents, but genetic variants in HLA genes influence HLA mRNA expression which might also affect antigen presentation. We evaluated the association between HLA gene polymorphisms and the risk for persistent HBV infection.

Methods: HLA gene polymorphisms were investigated in a case and control study of 294 chronic HBV carriers and 234 persons with HBV natural clearance by using a real-time polymerase chain reaction (RT-PCR).

Results: There was a significant association between the control and the case groups for rs9277535 allele frequencies ($P = 0.05$), but not significant for rs3077, in the Turkish subjects examined. Additionally, the AG haplotype block which showed a protective effect against the risk of persistent HBV infection (for the rs3077 A/rs9277535 G, OR = 0.52; 95 % 0.34–0.80, $P = 0.003$).

Conclusions: Our results demonstrate for the first time that HLA-DPB1 gene rs9277535 A allele, especially in male, has a major effect on the risk of persistent HBV infection, but not for rs3077. Further independent studies are necessary to clarify the association of these polymorphisms with persistence or natural clearance of HBV infection in Caucasian populations.

P-0357

Association between a polymorphism in microRNA target site of interleukin 16 and chronic hepatitis B

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Background: Interleukin-16 (IL-16) is an immunomodulatory cytokine, which plays an important role in some inflammatory and autoimmune diseases such as hepatitis B, which is a major health concern worldwide. In this study, we aimed to investigate the plausible association between IL-16 polymorphism and chronic HBV susceptibility in an Iranian population.

Methods: In a case-control study, we analyzed rs1131445 polymorphism in the microRNA binding site of the IL-16 gene in 262 patients with chronic hepatitis B and 269 healthy controls, using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method and DNA sequencing technology to confirm our results.

Results: Altogether, in this investigation, a significant association was observed between the IL-16 TC genotype compared with the TT genotype (OR = 0.696, 95 % CI 0.485–0.997, $P = 0.048$), after adjustments for confounders including age and gender.

Conclusions: These findings show that immunogenetic factors, such as single nucleotide polymorphism in IL-16, could be a risk factor for susceptibility to chronic HBV infection. However, further investigations are needed to verify these results.

P-0358

Japanese genetic study could not suggest the importance of the NTCP locus for HBV persistence

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Aim: Sodium taurocholate cotransporting polypeptide (NTCP) was recently identified as a hepatocyte receptor for hepatitis B virus (HBV). It has been reported that the amino acid substitution S267F, which corresponds to a single nucleotide polymorphism (SNP) rs2296651, of NTCP reduces taurocholate transporting activity and is defective in HBV receptor function. On the other hand, there are many other SNPs around the NTCP locus. We investigated the importance of the NTCP locus in HBV persistence by focusing on SNPs using a case-control association analysis.

Method: We selected 15 tag SNPs including rs2296651 of a 100-kb genomic region on 14q24.2, 65 kb upstream and 10 kb downstream of the NTCP locus. Haploview was used to select tag SNPs with a pairwise $r^2 > 0.90$ and minor allele frequency > 0.01 . We genotyped these SNPs in 1586 Japanese patients with chronic HBV infection and 750 control individuals by Invader assay.

Result: We successfully genotyped all tag SNPs. We found that minor allele (T) frequencies of rs2296651 were around 1 % in both controls and chronic HBV patients, and that no significant difference of its frequencies between them in any genetic models. Finally, we found no significant association between all other SNPs and susceptibility to chronic HBV infection.

Conclusion: We could not find any genetic association between the NTCP locus and HBV persistence in Japanese population. Further genetic analysis in populations with higher frequency of rs2296651 would gain understanding about the role of NTCP in chronic HBV infection.

P-0359

Lack of association between IL-12RB1 gene polymorphism (rs11575934) and chronic hepatitis B

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Background: Hepatitis B virus (HBV) is a major public health problem with more than one third of world's population infected.

Successful clearance and elimination of the infection from the body or progression of HBV infection to chronic disease depend on the host genetic background in immune system genes. Interleukin-12 (IL12) and also Interleukin-12 Receptor B1 (IL 12 RB1) are the key factors in the spontaneous clearance of viral infections. The aim of present research is to investigate the association between Interleukin-12 receptor B1 gene polymorphism (rs11575934 A/G) and Susceptibility to chronic hepatitis B virus infection.

Methods: In this case control study, genomic DNA of 150 chronic HBV infected patients and 150 healthy controls who were referred to the Taleghani Hospital in Tehran was extracted by salting out method. Single nucleotide polymorphism (rs11575934 A/G) was genotyped using polymerase chain reaction/restriction fragment length polymorphism (PCR-RFLP).

Results: The frequency of (rs11575934 A/G) gene for GG, AG, AA genotypes was (6.7, 40.7, 52.7 %) in chronic patients and (12.7, 41.3, 46 %) in control group respectively. After stages of genotyping and statistical analysis, No statistically significant difference between case and control groups has been observed ($p = 0.176$).

Conclusion: In the present study, no significant relation between (rs11575934 A/G) single nucleotide polymorphism of the IL12RB1 gene and susceptibility to chronic hepatitis B virus infection has been observed. According to the study, this polymorphism in the IL12RB1 gene does not affect the susceptibility to chronic HBV infection.

P-0360

Polymorphisms of interleukin 12 gene are not associated with chronic hepatitis B in Iranian patients

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Background: HBV infection is a major cause of chronic liver diseases worldwide. IL-12 is key regulatory cytokine for the secretion of IFN- γ by Th1 cells and natural killer cells. Genetic variations in cytokine genes can lead to changes in cytokines production. IL-12 induces development of Th1 cells and IFN- γ production which promote viral clearance. The objective of this study was to examine the possible roles of variations in IL12A and IL12B genes in the development of chronic hepatitis B (CHB).

Material method: A total of 187 patients and 187 healthy controls were enrolled in this study. Polymorphisms at positions -1148T/C (rs2243123) and 277G/A (rs568408) in the IL-12A gene and rs6887695G/C in the IL-12B gene were determined using polymerase chain reaction-restriction fragment length polymorphism method.

Result: No association was found between polymorphisms in IL12 gene and individuals' susceptibility to CHB. The frequencies of rs2243123 TT, TC and CC genotypes in the patients with chronic infection were 72.4, 24.8 and 2.9 % respectively and in healthy controls were 68.1, 26.2, 5.7 % (P value = 0.307) and frequencies of rs568408 GG, AG and AA genotypes in the patients were 68.1, 29.5 and 2.4 % and in controls were 70.5, 26.7, 2.9 % ($P = 0.789$) and frequencies of rs6887695GG, GC and CC genotypes in the patients

were 54.8, 38.1 and 7.1 % and in controls were 47.6, 45.2, 7.1 % (P value = 0.312).

Conclusions: These results revealed for the first time that there is no association between genetic polymorphisms of IL12 gene (rs2243123, rs2243123 and rs6887695) and development of CHB in Iranian population.

P-0361

HLA DPB1*15:01 predicts spontaneous HBsAg seroconversion in chronic hepatitis B

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Introduction: Chronic hepatitis B is an immunologically driven disease. The virus itself is non-cytopathic and the course of the disease is determined by host immunogenetic factors. Recent GWAS revealed significant associations of hepatitis B infection chronicity and HLA class II gene region near DQ and DP. Here we aimed to genotype DPB1 alleles in our cohort.

Methods: Ninety four chronic hepatitis B patients who were followed up at hepatology clinic and, age and gender matched 85 HBsAg negative, anti-HBs positive and anti-HbcIgG positive spontaneous seroconverted healthy subjects were enrolled. Genomic DNA was extracted from peripheral blood samples. DPB1 alleles were determined by restricted fragment length polymorphism at medium resolution. The distribution of the alleles among patients and control subjects were analyzed. Randomly selected 50 samples sequenced for external control. Sequence analysis completely matched with RFLP results.

Results: The patient group was consisted of 58 male (61.7 %) and 36 female. The mean age was 47.9 ± 14.7 , 71 (75 %) of them were HBeAg negative, 16 (17 %) had inactive disease. The pretreatment mean ALT, AST and log DNA were; 102.1 ± 113 U/L, 70 ± 96 U/L and 4.9 ± 2.8 IU/ml, respectively. Mean Ishak fibrosis score was 2 ± 1.5 and hepatic activity index was 6.5 ± 3.3 . Among the 19 analyzed DPB1 alleles in this study, DPB1*02:01 was the most prevalent (genotyped in 30.2 %). DPB1*15:01 was frequent in control group (15.3 vs. 1.1 %, $\chi^2 = 12.5$, OR = 0.06, 95 % CI = 0.08–0.046, $p < 0.001$, $pc < 0.001$). DPB1*02:01 and DPB1*10:01 were genotyped more frequently in the control (38.8 vs. 22.3 % $p = 0.02$ and 16.5 vs. 5.3 % $p = 0.02$, respectively).

Conclusion: HBsAg seroconversion is the ultimate target in hepatitis B. This study revealed an association of DPB1*15:01 allele with spontaneous HBsAg seroconversion.

P-0362

The clinical difference of HBeAb-positive inactive carrier among medical facilities in Japan

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Background: The investigation of HBV carriers has been conducted by high-volume center for hepatitis in Japan. In this study, we examined the differences in the clinical background of HBeAb-positive inactive carrier between the regional core centers for the treatment of liver disease (regional core centers) and the general hospital in Japan.

Methods: Inactive carrier was defined by The Japan Society of Hepatology. HBeAb-positive HBV carriers with normal liver function in four regional core centers for hepatitis and eight general hospitals were statistically compared about age, gender, Genotype, HBsAg amount, HBV DNA amount, and the presence of hepatocellular carcinoma.

Results: Three hundred fourteen cases in regional core center, and 165 cases of general hospital were analyzed. The number of HBV genotype were 15/45/144/1/16/93 (A/B/C/D/not detected/data unknown) in regional core center, and 7/43/68/0/47/0 (A/B/C/D/not detected/data unknown) in general hospital. The distribution of genotypes in several facilities had showed a significant difference ($P < 0.001$, chi-square test). The HBsAg level were 1.98 ± 1.76 logIU/ml in regional core center, and 1.68 ± 1.82 logIU/ml in general hospital ($P = 0.097$, unpaired t test).

Conclusions: This study showed the difference in clinical background of HBeAb-positive inactive carrier between the regional core centers and general hospital.

P-0363

Are we missing out some of chronic hepatitis B patients who actually needs treatment?

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Background: Current guidelines recommend to follow up patients with HBeAg negative chronic hepatitis B (CHB) infection whose HBV DNA levels are between 2000–20,000 IU/ml and have persistently normal alanine aminotransferase (ALT) concentration (PNALT). In this study we investigate liver inflammation and fibrosis score in these patients with liver biopsy.

Methods: Ninety HBeAg negative CHB patients with PNALT and serum HBV DNA levels between 2000–20,000 IU/ml were retrospectively evaluated with liver biopsy from 2012 to 2015 April. PNALT is described as ALT levels below the upper limit of normal (ULN) measured by 3 months intervals at least for 1 year.

Results: Basic demographic profile, liver histology, ALT, HBV DNA levels at the time of biopsy are summarized in Table 1.

Conclusions: HBV DNA levels and ALT concentrations enhance liver damage and HCC explicitly. It is recommended to follow up PNALT patients with HBV DNA levels between 2000–20,000 IU/ml as inactive chronic HBV carriers. But results of this study show that moderate and severe fibrosis nearly in the half of the patients. To follow up these patients without therapy can increase risk of liver damage and HCC. For these reasons we recommend performing biopsy and to assess need of therapy according to the biopsy result in the group of patients HBeAg negative CHB with HBV DNA levels between 2000–20,000 IU/ml and PNALT.

Patients Characteristics at time of biopsy	n = 89
Age (y)	34.9 (18-73)
Female n (%)	56 (62%)
Necroinflammation grade	
Minimal-Mild (0-8)	58 (64,4%)
Moderate-Severe(9-18)	32 (35,6%)
Fibrosis stage	
No/mild (0-1)	44 (48,9%)
Moderate (2-3)	42 (46,6%)
Severe (4)	4 (4,4%)
ALT	28 (12-55)
Serum HBV DNA IU/ml	6265 (2,276-14,227)

Table1. Demographic and Clinical Characteristics of Patients

P-0364

The prognosis of hepatitis B inactive carrier in Japan: a multicenter prospective study

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Background: Negative hepatitis B e-antigen (HBeAg) inactive carriers that has been defined in several guidelines, are considered good prognosis. In this study, we conducted prospective study with 14 faculties in order to clarify the prognosis of negative HBeAg inactive carriers (IC).

Methods: Three hundred fifty-eight IC cases at baseline were prospectively observed from January 2011 to November 2014. The IC criteria was defined by Japan Society of Hepatology. We evaluated the primary endpoint defined as exceeded ALT, HBV DNA or both levels during the follow-up period. Also, we analyzed the development of cirrhosis, Hepatocellular carcinoma (HCC) or liver-related death.

Results: At baseline, the mean age was 57.1 ± 13.3 years. During the follow-up period (were 1025 ± 235 days), no one had developed cirrhosis, HCC or liver-related death. The number of exceeded ALT levels were 35 cases (9.8 %), and exceeded HBV DNA levels were 34 cases (9.5 %). Factors associated with deviation of the IC criteria, in the multivariate analysis, were ALT, HBV DNA, γ -glutamyl transpeptidase (γ -GTP) at baseline.

Conclusions: The majority of IC cases in Japan had good prognosis. However, despite the observation in a short period of time, not less cases had deviated from IC criteria.

P-0365

Febrile neutropenia associated with hepatitis B infection: a case report

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Aim: This case aims to present febrile neutropenia complication, seldomly encountered during extended hepatitis B.

Case: The 45 years old female patient applied to the emergency service of our hospital with fever, icterus and joint pain complaints. From the anamnesis, it was determined that the patient had applied to an hospital with same complaints and had been diagnosed with acute hepatitis B. Since her bilirubin levels did not decrease and neutropenia had developed, she was sent to our hospital. Icterus in sclera and erythematous skin rashes on the body had been identified in the physical examination of the patient having 39 °C of body temperature. The patient having AST: 211 mg/dl, ALT: 238 mg/dl, total bilirubin: 32.7 mg/dl, PNL: 204/mm³ was hospitalized. Positive viral markers; HBsAg (+), Anti HBc IgM (+), AntiHBc IgG (+), Anti HBe (+). Granulocytic series cells and precursors were not observed at the examination of peripheral smear, bone marrow aspiration and biopsy. Granulocyte colony-stimulating factor (GCSF) was started. The fever and neutropenia were continuing on the 16th day of the GCSF treatment. By taking serum sickness and bone marrow suppression associated with hepatitis B into consideration, lamivudine was added for treatment. On the 4th day of the lamivudine treatment, the fever of the patient returned to normal and she recovered from neutropenia. While continuing lamivudine treatment, the patient was discharged from the hospital.

Conclusion: This case emphasizes that in community-acquired febrile neutropenia, hepatitis B should be kept in mind even rarely encountered.

P-0366

Strategies to improve utility of chronic hepatitis B therapy based on questionnaire survey in Japan

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Aim: Since the current anti-viral therapy for chronic hepatitis B (CHB) is far from complete cure, patients have been compelled to continue the medical care eternally. Since 2012, Japanese Government has funded enormous grant for the research to develop novel drugs eradicating hepatitis B virus (HBV), as the result of reconciliation of the lawsuit concerning HBV spread due to needle sharing in vaccination. Thus, we aimed to explore the contributing factors to improve the utility of anti-viral treatment for CHB and to analyze the patients' desires for the ongoing research in Japan.

Methods: A questionnaire survey, including 126 questions regarding attitude for experienced therapies such as interferon and nucleoside analogues and desires for drug development, was performed in 63 core-center hospitals in Japan from Aug/2013–Jan/2014.

Results: A total of 3021 answers to the questionnaire were collected. In decision tree models using 333 variables generated from the questionnaire choices, satisfaction and resolution of anxiety in experienced therapies were dependent on effects of treatment, sufficient information provided by physicians and less disturbance of daily life including job. Medical expenses were not selected at superior branch because of subsidy for anti-viral therapy started from 2010. In analysis of free descriptive answers regarding desires for treatment and supports, fewer disturbances of daily life and mental pain due to infectious disease as well as expectation for the ongoing research were variously selected according to patients' attribute.

Conclusions: The patients' desires should be considered in drug development and clinical practices, together with favorable effects of treatment.

P-0367

The paths to diagnosis in HBsAg positive individuals

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Objective: Hepatitis B virus is transmitted by various routes, and the infections usually cannot be detected during an acute phase due to asymptomatic course of the infection. The aim of the present study is to determine the paths that lead to the diagnosis in HBsAg-positive individuals.

Method: Using face-to-face interview technique, a questionnaire evaluating the paths to diagnosis was administered to HBsAg-positive cases, who were followed in Viral Hepatitis Outpatient Clinics at Izmir Bozyaka Education and Research Hospital.

Results: The study included a total of 951 HBsAg-positive cases who were aged 17–84. When the study data were analyzed, 153 cases (19 %) did not remember the path to the diagnosis.

Conclusion: Due to the fact that HBV infection is often asymptomatic and infected people do not realize they are infected, various paths lead to the diagnosis in HBsAg-positive individuals. The present study suggested that the majority of the people with HBV infection is detected during routine screening tests performed for other reasons, and this is followed by blood donation, premarital screening tests, blood tests in the pregnancy period. This is followed by blood tests performed due to the presence of HBsAg-positive individuals in the family. Routine screening of the individuals for HBV infection should not be neglected and the individuals must be provided information on this subject.

P-0368

Evaluation of HBV infection status in family members of HBsAg-positive individuals

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Objective: The evaluation of HBV infection status in family members of HBsAg-positive individuals should not be neglected due to vertical and horizontal transmission risk for hepatitis B virus. The aim of the present study was to evaluate the HBV infection status in the mothers, fathers, and siblings of HBsAg-positive individuals.

Method: Using face-to-face interview technique, a questionnaire was administered to HBsAg-positive cases who were followed in Viral Hepatitis Outpatient Clinics at Izmir Bozyaka Education and Research Hospital in order to evaluate HBsAg positivity in their mothers, fathers, and siblings, and the results were recorded if tests were available.

Results: The study included a total of 895 HBsAg-positive cases who were aged 18 to 82 years. The mothers of 888 (82 %) cases were never tested for HBV infection. The rates for the mother of HBsAg-positive cases was (51 %), fathers (31 %) and the siblings (79 %). The rate of died family members due to cirrhosis and two (3 %) due to HCC was 6.7 % in the mothers, 17.9 % in the fathers, and 4 % in the siblings.

Conclusion: The analysis of the data suggested that the rate of testing for HBV infection was extremely low in the mothers, fathers, and siblings of HBV-infected individuals. Also these data suggested that the vertical transmission of HBV is the most important transmission way in our country. Therefore mothers, fathers, and siblings of the individuals showing HBsAg positivity must be tested for the markers of HBV infection and the testing of these individuals should not be neglected.

P-0369

Multiple failures: addressing the barriers to hepatitis B treatment in China

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China has the largest absolute number of people in the world infected with hepatitis B. While hepatitis B treatments are effective in reducing disease progression, only a small minority of people with hepatitis B in China access these treatments. This project aimed to identify the systematic barriers to hepatitis B treatment by documenting the personal impact of the infection. The study used a qualitative methodology with people with chronic viral hepatitis in four Chinese cities during April 2014 and other key service providers, with interview data systematically reviewed to identify key issues, concepts and themes. Participants identified multiple barriers to treatment and treatment services. These are framed by non-systematic diagnosis processes where testing occurs through educational institutions or workplaces by staff without health expertise leading to a poor or incomplete understanding of the infection. While most participants monitored their infection, treatment choices were determined by economic access with the lack of public funding for treatments having a substantial individual, social and economic impact, particularly when several family members are affected by viral hepatitis. Treatment compliance was affected by economic issues and lack of information and knowledge. Issues of disclosure were noted by many participants particularly in the context of intimate relationships and within workplaces, with the fear of disclosure often limiting career and treatment choices. Hepatitis B treatments are only useful when people with hepatitis B access these treatment. Identification of the barriers to treatment and treatment services provides government and other service providers with guidance for improving treatment access.

P-0370

Sero-epidemiological study of hepatitis viral infections among 2105 employees in Hiroshima, Japan

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Background: In Japan, hepatitis virus screening for residents over 40 y.o. started from 2002. However, the coverage rate of hepatitis virus screening among employees is still low (<20 %), and the prevalence of HBcAb is not clear among employees. The aim of this study is to clarify the characteristics of hepatitis virus infections among employees.

Method: Serological testing and questionnaire survey were conducted among employees from 11 companies in Hiroshima from 2010 to 2013. HBsAg (RPHA), HBsAb and HBcAb (CLIA), Anti-HCV (PA), HCVcAg (ELIZA), and HCV RNA (RT-PCR) were determined.

Results: Overall, 2105 employees (1666 males, 439 females; 49 ± 15.1 years) were enrolled in this study. The coverage rate of hepatitis virus screening was 13.3 %, being lower as compared to that of the residents (26.6 %). Prevalence of HBsAg, HBcAb, HBsAb and HCV RNA were 1.05, 16.3, 14.4, and 0.48 %, respectively. Prevalence of HBcAb was high (40 %) at the 50s–60s and that of HBsAg and HCV RNA were peaked at the 70s (2.9 %) and the 60s (1.31 %), respectively. Among carriers who were positive for HBsAg or HCV RNA, 15.7 % had not visited hospitals because they thought that it was unnecessary. After starting to send referrals to all carriers, 53 % visited hospitals.

Conclusion: The coverage rate in this study was low, and prevalence of HBcAb was high at the 50s–60s. This survey found that not all carriers visit hospitals even after they were found to be positive. It is worthy to increase its coverage rate among employees and motivate positives to visit hospitals.

P-0371

Clinical characteristics and management status of hepatitis B: a cross-sectional study

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Introduction: Hepatitis B infection is serious health problem in Pakistan. In view of the serious socioeconomic consequences of HBV infection, identifying patient characteristics and current treatment practice for this disease will enhance regulation of their medical management. The present study was designed to provide real-life data on HBV infection in an effort to improve the quality of treatment and public health practice in controlling the disease.

Methods: We undertook an observational, cross-sectional, epidemiological study at the Jinnah Postgraduate Medical Centre, Karachi during the period of January 2014 to September 2015. Male or female patients of any age and had documented hepatitis B were eligible for inclusion in the study. Hepatitis B infection was defined as a positive hepatitis B surface antigen test. Data collected from the case report form included demographic information, comorbidities, concomitant infections, the most recent laboratory tests and results, the presence of hepatic complications, previous and current antiviral treatments taken.

Results: A total 144 patients were analyzed. The median duration of documented infection was 6 years in HBV-infected patients. Upper gastrointestinal bleeding was the most frequent hepatic complication (14.6 %). Antiviral medications had been received by 41 % of patients. Nucleos(t)ide analogs (27.2 %) were the major antiviral

medications prescribed for HBV-infected patients (most commonly entecavir).

Conclusion: This observational, real-life study has identified some gaps between clinical practice and guideline recommendations in Pakistan. To achieve better health outcomes, several improvements, such as disease monitoring and optimizing antiviral regimens, should be made to improve disease management.

P-0372

Are hepatitis patients hiding their disease?

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Objective: Some of the chronic hepatitis patients are hiding their disease from other people. In this study, we aimed to determine if some of the chronic hepatitis patients are hiding their disease from other people.

Method: Using face-to-face interview technique, a questionnaire was administered to HBsAg-positive cases who were monitoring in Viral Hepatitis Outpatient Clinics at Izmir Bozyaka Education and Research Hospital.

Results: The study included a total of 378 HBsAg-positive cases who were aged 20 to 76 (220 female, 158 male). In total, 76 patients (20 %) were hiding their disease from their neighbours and work friends, especially employers. We found that university graduates were more likely to hide their condition compared to elementary school graduates and dropouts. We have also found that the university graduates are more anxious about their condition being revealed to their employers, as this may cause them to lose their jobs. There is a relationship between education level and hiding their disease condition. This relation was found statistically significant ($P < 0.00001$, Chi-square: 37.8807). Most of the patients afraid of isolating or being insulted from the society.

Conclusion: In this study we have found that one fifth of patients were hiding their condition from the immediate surroundings. The reason of this condition is related to inadequate knowledge about the transmission and prevention ways of viral hepatitis.

P-0373

Differentiation in patients with acute hepatitis B and chronic hepatitis B with acute exacerbation

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Background/Aims: In endemic area of hepatitis B infection, it is difficult to distinguish acute hepatitis B (AHB) from chronic hepatitis B with acute exacerbation (CHB-AE) due to the similar serological profiles and clinical features. This study aimed to investigate clinical, biochemical and virological differentiation in AHB and CHB-AE.

Methods: A total of 59 patients with IgM HBcAb seropositivity from January 2005 to December 2014 were enrolled. The subjects were divided into the AHB group ($n = 40$) and the CHB-AE group ($n = 19$) according to previous history of hepatitis B infection or radiologic examination. Clinical, biochemical and virological features were analyzed and compared between the both groups retrospectively.

Results: Presence of jaundice and HBeAb seropositivity in the AHB group were significantly higher than those in the CHB-AE group (72.5 vs. 42.1 %; $p = 0.042$ and 60.0 vs. 26.3 %; $p = 0.002$, respectively). Levels of serum HBV DNA significantly differed between the AHB group and the CHB-AE group (4.9 vs. 6.7 \log_{10} IU/ml; $p = 0.000$). In addition, levels of serum alpha-fetoprotein significantly differed in the two groups (5.5 vs. 135.5 ng/ml; $p = 0.001$). However, no significant difference in seropositivity rates of HBsAg and HBeAg was observed between the both groups (90.0 vs. 100 %; $p = 0.294$ and 55.0 vs. 78.9 %; $p = 0.133$, respectively). Moreover, levels of HBsAg cut-off value [S/CO] was not significantly different between the two groups (2041.2 vs. 2078.6; $p = 0.756$).

Conclusions: This study showed that the presence of jaundice and HBeAb seropositivity as well as the levels of serum HBV DNA and alpha-fetoprotein might be used to distinguish from AHB and CHB-AE.

P-0374

Hep B prevention

Amrish Mishra

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Introduction: Hepatitis B virus (HBV) can have serious consequences and in some cases fatal where a person already has chronic liver disease. This study estimates the needle/syringe sharing habits and prevalence of BBV transmission depending on marital status of IDUs.

Methods: A cohort 309 active IDUs (23 females) from 11 needle/syringe program site within Kathmandu were screened for HIV, hepatitis B and C. Structured questionnaires were used to collect behavioral data relating to sexual practices: which were assessed as protected or unprotected sexual practice. Data on drug injection and needle/syringe sharing habits (preused syringe, syringe given by others or use of syringes by self or others that are kept in public places) which were assessed in terms of their last three recent injections. Quantitative data was analyzed via SPSS 13.0 using descriptive and inferential statistics and the study was done from Dec 2014 to September 2015.

Results: Among the 309 clients, the prevalence of HBV was 5 % (14), HCV 66 % (203) and HIV 10 % (32). The prevalence of BBV differed significantly according to marital status, IDUs who were married (43 %) were nearly three times more likely to carry HIV, HCV or HBV. HBV immunization was found to be significantly low at 6 %. Data on high risk needle/syringe using behavior indicated: most recent injection 0.9 % Second most recent 0.5 % and Third most recent 0.8 %, indicating nearly one percent was practicing high risk injecting behavior.

Conclusion: HBV immunization practices is highly recommended to those groups that are at high risk of hepatitis B infection should be vaccinated.

P-0375

To determine the prevalence of hepatitis B disease stages

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Objective: To determine the prevalence of hepatitis B disease stages in different age groups of the patients which first visited at Asian institute of Medical Science hospital Hyderabad.

Material and methods: This observational and descriptive study was conducted at AIMS Hospital Hyderabad during the time of January 2010 to December 2012. In this study all patients' presentation was noted on the Performa who first visited with HBV, all age groups were included in this study.

Results: All patients of this study mostly infected with HBV were with young age group with the percentage of 32.25 % between the 26–35 year. Mostly patients was found in HBV phase of chronic hepatitis inactive carries. HCV was found in the patients of this study very poor.

Conclusion: We concluded in this study according to hepatitis B phases, mostly patients were found in chronic hepatitis inactive phase and very poor percentage of the patients with HCV positive in the HBV infected patients.

P-0376

Stigma, discrimination and living with hepatitis B in Turkey

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In our country, the estimated number of people with chronic HBV infection was 3,718,889 (% 5) in 2013. This study aimed to examine the prevalence of stigma among individuals with hepatitis B and the association between stigma and secrecy in Turkey. Voluntary written questionnaires were administered to patients with hepatitis B. Data analysed with SPSS 20.0 package program. Structural equation modeling (SEM) was used to examine the associations between secrecy, awareness, level of anxiety and social relationships. 56 % of the 505 participants were male. 20 % of the participants stated that they felt embarrassed about their hepatitis B status, 10.5 % didn't share with anyone and 36.6 % shared only with people very close to them. 12.5 % of the patients stated that their sexual life was negatively affected, 12 % of them weren't considering to get married because of their illnesses. 10.3 % of the patients stated that the health personnel behaved timidly to them. 21.6 % of the patients had problems while getting health service and didn't feel comfortable. 17 % of participants stated that they were affected negatively in financial terms. 63 % of the participants had HBsAg positive people in their families. 61.8 % of the participants stated that they hadn't even heard about the name of the illness before they got ill. Only anxiety was found to be significantly effective on the association with SEM. Participants of this study were often reluctant to disclose their hepatitis B status for fear of being stigmatized. As a conclusion, these results suggest that hepatitis B associated self stigma is a problem in Turkey.

P-0377

China on the Scheldt: HBV and HCV in the Chinese population in Belgium and study of screening methods

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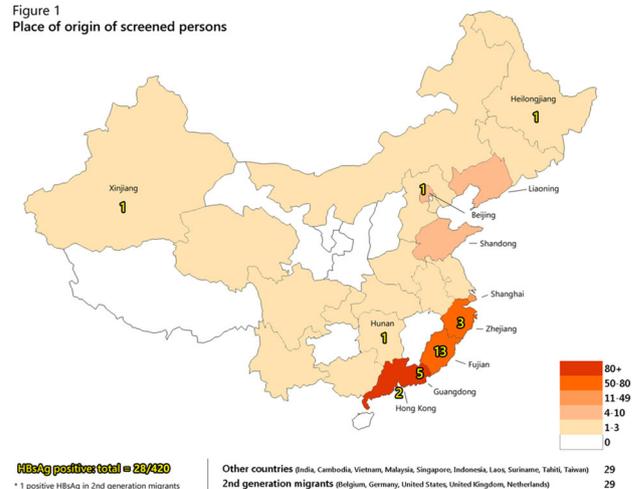
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Background: Belgium has a low hepatitis C virus (HCV) and hepatitis B virus (HBV) endemicity rate. Recent reports suggest a considerable fraction of the viral hepatitis burden in Western countries is introduced by immigrants from high endemic countries such as China. This study aims to screen for HBV and HCV infection by testing HBsAg, anti-HBc and anti-HCV in the Chinese community in Belgium. Three screening methods are compared: serum, saliva and dried blood spot (DBS).

Methods: On-site screening with the cooperation of volunteers of the Chinese community was performed starting 25/10/2014 and is ongoing. Minimal clinical and personal information were obtained. Saliva testing was performed using the OraSure Intercept 2 Oral Collection Device. PBS-Tween 0.05 % solution was used to acquire eluates from DBS on Whatman Protein Saver 701 cards. All sample types were tested on the Roche Elecsys serology platform.

Results: 420 persons were screened (characteristics in Table 1). Serological testing: HBsAg was positive in 28/420 persons (6.6 %). Almost half were unaware of their HBV infection (15/28, 46.4 %). Anti-HBc antibody was positive in 222/420 persons (52.9 %). Nobody was positive for anti-HCV. Comparative serologic testing and validation tests of saliva and DBS are currently underway. 391 persons were born in China. Place of origin is shown in Fig. 1.

Figure 1
Place of origin of screened persons



Conclusions: HBsAg seroprevalence in Chinese immigrants in Belgium is high (6.6 %) and HBV status was unknown to many (46.4 %). More than half of screened persons (52.9 %) are anti-HBc positive. Most come from Eastern and Southern Chinese regions.

Table 1

Characteristics of screened persons

Age (median, years, spread)	52 (18–86)
Gender (female, %)	248/420 (59.0)
Country of origin (China, %)	369/420 (87.9)
Vaccination (%)	110/420 (26.2)
Previous/current treatment ¹ (%)	12/420 (2.9)
Access to primary care (%)	320/420 (76.2)

¹ Anti-viral therapy, interferon, traditional Chinese medicine

P-0378

The virological data of the patients with HBV-HCV co-infection

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Object: In order to investigate the virology and biochemistry situations of the cases with HBV-HCV co-infection.

Method: retrospectively analyzed the data of patients with HBV-HCV co-infection who had been followed up in the chronic hepatitis C clinics from 1st January 2008 to 30th June 2015 before they accepted anti-HCV therapy.

Results: 70 patients were observed, their ages were 40.54 ± 10.59 years, male: female was 47:23 in gender. 4 patients among the 70 patients had been received nucleoside analogs anti-HBV therapy in advance because the flare of Hepatitis B, after the treatment of anti-HBV, the patients' HCVRNA increased and caused the onset of hepatitis again, then they need anti-HCV therapy. 36 cases had been detected the HCV genotype, 23 cases were genotype 1b, 9 cases were genotype 6a, the rest were genotype 2a (1 case), 3a (1 case), and 3b (2 cases). The detection of HCVRNA was 6.04 ± 1.11 log, except the 4 cases who had received anti-HBV 52 cases had been detected HBVDNA, 14 cases were detectable, and 38 cases were undetectable. The HBVDNA which were detectable was 4.64 ± 2.05 log. 33 cases had the results of HBeAg detection, 8 (24.24 %, 8/33) cases were positive, while 25 (75.76 %, 25/33) cases were negative. HBsAg were 2.45 ± 0.16 log IU/ml, ALT 67.47 ± 43 U/L.

Conclusion: In the cases with HBV-HCV co-infection, HBV and HCV were showed virus interference. In our study, HCV predominated over HBV, HBVDNA load and the titer of HBsAg were low and less than 25 % of cases were HBeAg(+).

P-0379

Cross sectional study of prevalence and risk factors of hepatitis B and C infection in rural India

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Background: Hepatitis B virus (HBV) and hepatitis C virus (HCV) are among the principal causes of severe liver disease. There is

limited data of epidemiology of Hepatitis B in community, more so in rural population. This was a community based cross sectional study. 1833 randomly selected subjects from a rural area were interviewed for risk factors for transmission and tested for markers of hepatitis B and hepatitis C infection. All the positive card tests were confirmed by ELISA.

Observation and Results: Out of 2400 subjects, rate for participation was 76.38 %. None of the subjects was positive for anti HCV antibody. Point prevalence for HBsAg positivity was 0.92. Being healthcare worker and having tattoo were significantly associated with HBsAg positive results. Nose and ear piercing was reported by almost. History of blood or blood product transfusion, I/V drug abuse, multiple sexual partners, unsafe Injections, hemodialysis and any h/o surgery was not associated with HBsAg positivity.

Conclusion: This study underscores the importance of universal precautions in healthcare workers. There is need to educate people about risk factors and prevention of transmission of hepatitis B and C.

P-0380

The virological response of the patients with HBV-HCV co-infection after accepting anti-HCV therapy

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Objective: In order to investigate the effects of the patients with HBV-HCV co-infection after they had received the anti-HCV therapy.

Method: retrospectively analyzed the virological data of patients with HBV-HCV co-infection who had been followed up in the chronic hepatitis C clinics from 1st January 2008 to 30th June 2015 after they accepted anti-HCV therapy.

Results: totally 53 cases with HBV-HCV co-infection accepted anti-HCV therapy with α -interferon combined with ribavirine, all of them had finished the procedure of at least 24 weeks, and they reached the endpoint or not. The rapid virological response (RVR) was 83 % (44/53), early virological response (EVR) was 92.5 % (49/53), and 48 cases got to the endpoint of treatment, around 89.6 % (43/48) achieved end-of-treatment response (ETR). 26 cases accepted the followed-up after finishing the therapy, sustained virological response(SVR) was 73.08 % (19/26), 21.7 % (5/26)cases relapsed, non-responder with sustained positive HCVRNA was 7.6 % (2/26).

Conclusion: the cases with HBV-HCV co-infection had good tolerance in the treatment of α -interferon combined with ribavirine, RVR and EVR were satisfied, but according the results of long term followed up still had around 30 % of the cases with poor response(relapsed and non-responder).

P-0381

Impact of occult hepatitis B on the clinical outcomes of patients with chronic hepatitis C infection

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Purpose: To clarify the contribution of concurrent occult hepatitis B (OHB) to the progression of liver disease in patients with hepatitis C virus (HCV) infection.

Materials and Methods: We collected 263 chronic anti-HCV-positive patients who had resolved hepatitis B virus (HBV) infection (anti-HBc positive and HBsAg negative). OHB was evaluated by both in-house polymerase chain reaction (PCR) assay and commercial assay for serum HBV DNA. Clinical outcomes including development of liver cirrhosis, hepatocellular carcinoma (HCC) and liver-related deaths were compared between OHB positive and negative subjects.

Results: Seven patients (2.66 %) had confirmed OHB (positive for HBV DNA by PCR in at least two sub-genome); 48 patients (18.25 %) had possible OHB (positive for HBV DNA by PCR in only one sub-genome); and the remaining 208 patients (79.09 %) were negative for OHB (negative in all tested sub-genome). The clinical outcomes were similar among three subgroups of patients. Patients with confirmed OHB tended to have a higher level of serum anti-HBc.

Conclusions: OHB may not contribute to the development of adverse liver outcomes in patients with HCV infection.

P-0382

Patients with active HBV had worse clinical features than active HCV alone in dual HBV/HCV infection

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Background: Chronic HBV and HCV dual infection is not uncommon in endemic areas. The impact of individual virus on the clinical course is unknown.

Aim: We conducted a retrospective study to explore the impact of viral activity on the clinical features of HBV/HCV dual infection.

Patients and Methods: A total of 85 patients with chronic HBV/HCV coinfection were recruited. By the baseline viral loads, they were categorized into three groups: group I, active HBV (HBV DNA ≥ 2000 IU/L) and active HCV (HCV RNA >15 IU/ml) ($n = 18$); group II, inactive HBV (HBV DNA <20 IU/mL) and active HCV ($n = 60$); group III, active HBV and inactive HCV (RNA: undetectable) ($n = 7$). Age, gender, serum albumin, AST, ALT, bilirubin, platelet, HBV DNA, HCV RNA and status of cirrhosis were compared.

Results: The mean age (50.7, 53.2 and 49.0 years in group I, II and III, respectively) and gender were comparable. Compared to group II, patients with active HBV (group I and III) had significantly higher AST (196 and 546 vs 79 U/L, $p = 0.029$ and $p < 0.001$, respectively), ALT (301 and 607 vs 122 U/L, $p = 0.036$ and $p < 0.001$, respectively) and bilirubin (1.6 and 4.3 vs 0.9 mg/dL, $p = 0.039$ and $p < 0.001$). The rate of cirrhosis was significantly higher in group I and III than group II (39 and 57 % vs 15 %, $p = 0.044$ and $p = 0.023$, respectively).

Conclusions: The patients with active HBV had significantly worse clinical biochemical features and higher rate of liver cirrhosis than active HCV alone in dual HBV/HCV infection.

P-0383

The epidemiological characters of the patients with HBV HCV co-infection

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Object: In order to investigate the epidemiology of the patients with HBV-HCV co-infection.

Method: retrospectively analyzed the data of patients with HBV-HCV co-infection who had been followed up in the chronic hepatitis C clinics from 1st January 2008 to 30th June 2015.

Results: totally 1932 cases with chronic hepatitis C were followed up, 92(4.76 %, 92/1932) cases were HBV-HCV co-infection among them. Among the 92 cases, 53 cases (57.61 %, 53/92) were from Guangdong Province, 39 cases (42.39 %, 39/92) were from the other provinces around Guangdong Province. The patients with HBV-HCV co-infection were 28.42–53.64 years old (Avg. = 41.03), male: female was 68:24; 46 (50 %, 46/92) cases were with the history of blood or blood product transfusion; 1 case accepted long term hemodialysis; 20 cases had the history of intravenous drug abusing; 2 cases had the history of the unclean intravenous injection; 3 cases had the history of unsafe dental therapy; 2 cases got affected by occupational exposure, 1 cases by the sexual activity. 17 cases were unknown reasons. 50 cases accepted the genotype examination, 34 cases (68 %, 34/50) were genotype 1b, 11 cases (22 %, 11/50) were 6a, and the rest 5 cases were 2a, 3a, 3b and 6n.

Conclusion: in south China the patients with HBV-HCV co-infection were in older ages and most of them were male in gender, the major ways of getting infected with HCV were blood or blood product transfusion and intravenous drug abusing, and the major genotype of HCV were 1b and 6a.

P-0384

Sustained virological response in dual infection of chronic hepatitis B and C

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Background/Aims: Dual hepatitis B virus (HBV) and hepatitis C virus (HCV) infections are common in HBV or HCV endemic areas. The prevalence is around 10–20 % in patients with chronic HBV infection and 2–10 % of anti-HCV positive patients to have markers of HBV infection. The purpose of our study is to assess the effect of combined Peginterferon alpha and ribavirin therapy in patients co-infected with hepatitis B and C.

Materials and Methods: Total 1688 viral hepatitis B and C infected patients that were followed by a single centre between 1998 and 2015. There were 1217 CHB and 471 CHC. Patients were eligible for

inclusion in the study if they had a positive test for HCV antibody or HCV RNA and positive test for HBsAg or HBV DNA. All of patients were followed in every 6 months. In these visits we examined HBsAg, AntiHBs, HBV DNA PCR, Anti HCV, HCV RNA PCR, ALT, AST, total and direct bilirubin.

Results: There were 34 patients HBV + HCV coinfecting in 1203 HBV infected patients (2.6 %) and 7 HBV + HCV coinfection in 471 HCV infected patients (6.7 %). There were 5 HBV + HCV active, 16 active HBV + HCV inactive, 5 active HCV+ inactive HBV, 8 inactive HBV+ inactive HCV, and 1 patients had triple infection HBV + HCV + HDV. Among the patients that have HCV infection, 3 died because of cirrhosis and HCC, 1 died because of renal failure.

Conclusion: HBV and HCV coinfection was found 2 % in patients. Combination treatment with Peginterferon plus Ribavirine in HBV/HCV co-infected patients have similar results as in patients who have only HCV infection.

P-0385

Detection of TTV in Iranian patients with HBV or HCV mono-infection, HIV and HCV coinfection

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Background: Torque Tenovirus (TTV) is a non-enveloped DNA virus with a single-stranded, closed circular genome and belongs to Circoviridae family. Although TTV is prevalent in general population, there are still a lot of unanswered questions about the frequency and effect of TTV coinfection with other viruses. So the aim of this study is to investigate TTV infection frequency in hepatitis C virus (HCV) and human immunodeficiency virus (HIV) coinfecting patients and also in hepatitis B virus (HBV) or HCV mono-infected patients. **Methods:** A total of 316 Serum samples from 36 HIV/HCV coinfecting patients, 101 HCV mono-infected patients, 77 HBV chronic patients and 102 healthy controls were studied. Biochemical and serological markers were determined and Nested PCR was performed for detection of TTV DNA and the results were statistically analyzed. **Results:** TTV DNA was detected in 180 of 316 samples (56.9 %). Although, higher frequency of TTV infection was observed among HCV mono-infected patients (64.40 %) in comparison with HBV patients (58.4 %), HIV/HCV coinfecting patients (55.55 %) and healthy controls (49.02 %), the difference was not statistically significant (p value = 0.88).

Conclusion: Our results demonstrated that the frequency of TTV infection in patients is a higher than healthy controls. Interestingly the prevalence of TTV in HCV mono infection patients is higher than HBV infected and HIV/HCV coinfecting patients. It seems that TTV does not have a significant effect on liver functional tests and disease severity among patients.

P-0386

HBV-HIV co-infection, a case report

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Background: In a study performed in our country showed 40 % of HIV infected individuals encountered with HBV and 4 % of these people had chronic HBV.

Case: A 27 year-old male patient with the complaint of weakness, nausea and jaundice in the eyes, approved to the clinic to research the etiology of hepatitis. He had no co-morbid disease and no medication. 5 months ago, he had unprotected risky sexual intercourse. His laboratory tests were like HBsAg: (+), Anti-HBs: (–), HBeAg: (–), Anti-HBe: (+), Anti-HBc IgM and IgG: (+), Anti-HAV IgG: (+), Anti-Delta: (–), AST: 867 mg/dL, ALT: 1046 mg/dL, GGT: 88 mg/dL, ALP: 155 mg/dL, total bilirubin: 10.5 mg/dL, direct bilirubin: 8.12 mg/dL, WBC: 4080/mm³, HB: 12.4 g/dL, PLT: 32 × 10⁴ U/L. He was diagnosed acute hepatitis B flare up and HBV-DNA PCR test was performed. Meanwhile, Anti-HIV ELISA test was positive for the second time and Western Blot test was performed to confirm ELISA tests. HIV RNA and CD4 cell count were performed. Approximately 2 weeks later, his biochemical values were like, AST: 87 mg/dL, ALT: 184 mg/dL, T.bil: 2.9 mg/dL, D.bil: 2.5 mg/dL. HBV DNA level was 1 × 10⁷ copy/mL, HIV RNA level was: 4400 copy/mL, CD4 cell count was: 308/mm³. The patient was diagnosed HBV + HIV co-infection and Truvada (Tenofovir + Emtricitabine) + Kaletra (Lopinavir + Ritonavir) treatment were administered.

Conclusion: In the patients co-infected HIV/HBV with high HBV replication, the risk of cirrhosis fourfold increases. It should be kept in mind that the possibility of HIV + HBV co-infection can be frequent and if needed from all the risky patients the tests should be performed.

P-0387

The risk of HBV and HIV transmission in long-haul truck drivers in Egypt

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Introduction: In many countries Long-haul truck drivers and their commercial sex contacts (CCs) have been associated with the spread of blood born and sexually transmitted infections (STIs). However, there is no sufficient information about the Blood born and STI risk behaviors of these populations in Egypt in our study we tried to gather information about blood born and STI-related risk behaviors in the drivers in Tanta and Kafr El-Sheikh governorates.

Methods: Between march and october 2014, we conducted face-to-face unstructured and semistructured qualitative interviews at trucking venues, health department facilities, and a community-based organization to solicit information on sexual behavior and condom and illicit drug use especially intravenous drugs. The interviews were audiotaped, transcribed, reviewed for quality control, and then coded and analyzed.

Results: Fifty long-haul truck drivers completed the interview. The truck drivers were male with a mean age of 40 years. Data suggested risky sexual behavior and drug use (i.e., inconsistent condom use, illicit drug use including intravenous drug use, and the exchange of sex for drugs) that could facilitate STI/human immunodeficiency

virus (HIV) and hepatitis virus transmission. Results also showed a low knowledge about STIs and lack of access to general health care for both populations. Fortunately we found that all the drivers were seronegative for hepatitis B virus and human immunodeficiency virus. **Conclusions:** Additional studies are needed to further assess risk of development and prevention of blood born and sexually transmitted diseases in long-haul truck drivers.

P-0388

Co-infection of Hepatitis B Virus with HIV and viral characteristics in Zimbabwe

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Background: Chronic HBV and HIV co-infected patients are potentially more prone to liver related illness and death facilitated by HBV reactivation and drug related hepatotoxicity. This is particularly problematic in Southern-Africa where both viruses are endemic. Hence, we determined HIV/HBV co-infection prevalence and viral characteristics among pre-HIV-treatment patients in Harare, Zimbabwe.

Methods: This laboratory-based descriptive cross-sectional survey utilized 176 remnant plasma samples collected between June and September 2014 from consenting HIV patients (median age 35 (18–74)). CD4 count, ALT and HBV seromarkers were determined by Flow-cytometry, colorimetry and chemiluminescence assays respectively. Core promoter/precure and small S genes of HBV and partial *pol* gene of HIV were determined by direct sequencing.

Results: Out of 176 participants (65.7 % female), 19 (10.8 %) were positive for HBsAg. The incidence of HBsAg was higher in men than women ($p = 0.009$). Median CD4 count was lower in HBsAg-positive subjects than HBsAg-negative ones ($p = 0.016$). HBV DNA was detectable in 11 HBsAg-positive samples (median 3.46 log cp/ml (2.93–5.38)) with 7 being amplifiable then sequenced. All were subtype A1 without lamivudine resistance mutations but each had at least 1 BCP/PC mutation including G1888A (5/7), precure Kozak sequence (5/7), A1762T/G1764A (3/7). Two samples were positive for HBeAg. For HIV, of 164 isolates genotyped, all except 1 were HIV-1 subtype C. Sixteen (9.8 %) had at least 1 drug resistance mutation, mostly from women ($p = 0.092$) and Non-nucleoside reverse transcriptase inhibitor-related.

Conclusion: The prevalence of HBV/HIV co-infection was high in this population necessitating improved HBV screening and early treatment in HIV patients.

P-0389

Alarming high prevalence of HDV infection in Mongolia

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Introduction: Mongolia has one of the highest prevalence of hepatitis B and C. In clinical practice it is thought that HDV infection is on the rise. However, HDV infection was not formally studied in Mongolia. Aim of study: To study the prevalence of HDV infection in Mongolia.

Method and subject: Study subjects were chosen based on two-stage cluster random sampling method. Total of 1,158 subjects (20–70 ages) were enrolled in this study. 599 (43.1 %) of them men and 659 (56.9 %) female. All participants on-site tested for HBsAg using rapid tests. 5–10 ml of blood was drawn and sera were separated following a standard protocol. Rapid test positive tested subjects serum specimens were tested for HBsAg, anti-HD-Ab and HD-Ag by ELISA (Diasorin, Italy). All anti-HDV positive serum tested for HDV-RNA by RT-PCR.

Results: The overall prevalence of HBsAg among study subjects were 10.6 % (123/1158). From 123 HBsAg positive subjects 83 were tested positive for HD-Ab (67 % of HBsAg positive population and 7.2 % of total population) and 8 subjects were tested positive for HD-Ag (6.5 % of HBsAg positive population). From 83 anti-HDV positive tested serums, there was positive tested HDV-RNA in 51 serums. This mean is that prevalence rate of active HDV infection 4.5 % in total population.

Conclusion: Prevalence of HBV infection in adults is high rate in Mongolia. HDV infection is alarmingly high in Mongolian population. It indicates that there is an urgent need for concerted action from all stakeholders within the Mongolian healthcare system.

P-0390

Prevalence of HDV in Chinese active CHB is low, with similar clinical profiles as non-HDV patients

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Background: The prevalence of HDV infection in Chinese population had some reports, however the HDV co-infection in active CHB (chronic hepatitis B) who should be treated was unknown.

Patients and Methods: This trial was a national study to enrolled active naive CHB patients (HBV-DNA $>2 \times 10^3$ IU/ml) from 25 medical centers. We determined the HDV antibody of serum sample in baseline by simultaneous competitive ELISA assay.

Results: This interim analysis included 710 CHB cases (278 advance fibrosis patients and 432 cirrhosis). Six patients were anti-HDV positive, and the prevalence rate in our group was 0.8 %, that was low level compared to before reports (0.8–13 %), the difference may be related with survey population, as we know the prevalence of HDV co-infection is high in HIV infection and injection drug users. In the

HDV co-infection patients, two were from northeast of China (Jilin province), two North China (Beijing) and two East China (Shanghai), and two were non-cirrhosis and four were cirrhosis. The clinical profiles had no difference between HDV co-infection patients and non-infection, such as demographics data (age, gender), liver functions (ALT, AST, ALB, bilirubin, prothrombin time), HBV virology index (HBV-DNA 4.6 ± 1.5 vs 5.3 ± 1.5 log IU/ml, HBsAg 2206 vs 1889 IU/ml). Conclusions: The prevalence of HDV infection in Chinese patients active CHB is low, and HDV co-infection seem to have limited impact on disease progression.

P-0391

Diagnostic importance of ccc DNA in patients with chronic hepatitis D

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Background and Aim: Current methods of antiviral therapy inhibit genomic replication of hepatitis B virus, but can be ineffective as they do not directly affect the nuclear covalently closed circular DNA (cccDNA). Some resources show occult hepatitis B being diagnosed in 6–7 % of patients with HBV. In chronic hepatitis D the replication of HBV DNA is suppressed by replication of HDV, however HBsAg amount should remain intact. The literature data on this is controversial. The aim of our study was to estimate the prevalence of occult HBV (HBsAg-) in patients with chronic HDV.

Methods: We have examined plasma and liver biopsy from severe 19 patients admitted to the hospital of Research Institute of Virology. The liver biopsies of all these patients were examined for cccDNA by standard methodology.

Results: Out of 19 patients with chronic HDV infection, confirmed by detection of anti-HDV IgM, 6 (32 %) were HbsAg-negative, whereas cccDNA was found in liver biopsies.

Conclusions: According to literature data the prevalence of occult hepatitis B in patients with chronic mono-HDV infection is less 10 %, we can assume prevalence of occult hepatitis (HBsAg-) among our patients three fold higher. The practical importance of these findings can be high. Even in HDV hyper endemic regions (Uzbekistan), the donor blood is not tested for HDV markers, which can be positive in the absence of HBsAg. Therefore, any blood transfusion to HBV patients may contain potential risk of HDV infection even with blood negative for HBsAg and HBV DNA.

P-0392

The HDV infections among injecting drug users with and without HIV infection in Taiwan

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Introduction: In Taiwan, injecting drug use has been the main route of transmission of the human immunodeficiency virus since 2005, and HBV, HDV and HIV have similar transmission routes. This has become an important public health issue. The aim of this study is to explore the conditions of HDV infections between injecting drug users (IDUs) with and without HIV infection in Southern Taiwan.

Materials and Methods: In this study, we enrolled 87 IDUs, including 27 anti-HDV seronegative IDUs and 60 anti-HDV seropositive IDUs, and also analyzed the results of liver function tests, CD4 cell counts, anti-HIV and HIV RNA.

Result: The prevalence of anti-HDV seropositivity among HBsAg seropositive IDUs was 68.97 % (60/87) in this study, and prevalence of anti-HDV seropositivity among HBsAg seropositive were 84.21 % among IDUs with HIV infection and 40.0 % among IDUs without HIV infection. Anti-HIV seropositivity was related to anti-HDV seropositivity (OR = 9.34, 95 % confidence interval = 2.67–31.59, $P < 0.001$). No significant difference between CD4 cell count, HIV RNA viral load and anti-HDV was noted in this study.

Conclusions: The prevalence of HDV infection among IDUs is higher than the non-IDUs, and due to anti-HDV seropositivity being significantly related to anti-HIV seropositivity, HDV infection among IDUs is still important. We suggest that for IDUs, HBsAg and anti-HDV should be monitored closely.

P-0393

Prevalence of anti-HDV in patients with chronic hepatitis B in Bafra county, Samsun province, Turkey

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Introduction: In Turkey the prevalence of HDV varies by regions between 2.5 to 45 %. In this study we investigated anti-HDV seroprevalence and epidemiological features among HBsAg carriers attending the outpatient clinic at Medibafra Hospital.

Methods: Totally 271 HBsAg positive individuals were tested for the presence of anti-HDV antibodies by enzyme immunoassay (EIA) between January 2009 and June 2015. The patients found positive for anti-HDV were tested for the presence of HDV RNA by polymerase chain reaction (PCR) method.

Results: Of the 271 HBsAg positive individuals tested for the presence of anti-HDV antibodies, 58 were male and 42 % were female. The mean age was 45.6 (18–75 years). Among the HBsAg positive patients, four (1.5 %) were positive for anti-HDV. Three of the patients found to be positive for anti-HDV were inactive hepatitis B carriers and were negative for the presence of HDV RNA. One patient with positive anti-HDV had chronic hepatitis B and was positive for the presence of HDV RNA.

Discussion: In a recent large real life cohort from Turkey involving different regions which included 7366 HBsAg positive patients it was reported that the positivity for anti-HDV was 2.8 %. In our study the prevalence of anti-HDV was found to be almost half of the average prevalence of the country. However we may conclude that as the morbidity and mortality of chronic delta infection is higher than chronic hepatitis b, all patients with HBsAg positivity should be routinely screened for the presence of anti-HDV.

P-0394

Epidemiology of HBV subgenotypes D**Fehmi Tabak, Resat Ozaras, Mucahit Yemisen, Ilker I Balkan**

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The natural history of hepatitis B virus infection is not uniform and affected from several factors including, HBV genotype. Genotype D is a widely distributed genotype. Among genotype D, several subgenotypes differentiate epidemiologically and probably clinically. In this study, we reviewed the medical literature reporting subgenotypes of genotype D and aimed to explore the epidemiology of subgenotypes. D1 is predominant in Middle East and North Africa, and characterized by early HBeAg seroconversion and low viral load. D2 is seen in Albania, Turkey, Brazil, western India, Lebanon, and Serbia. D3 was reported from Serbia, western India, and Indonesia. It is a predominant subgenotype in injection drug use-related acute HBV infections in Europe and Canada. D4 is relatively rare and reported from Haiti, Russia and Baltic region, Brazil, Kenya, Morocco and Rwanda. Subgenotype D5 seems to be common in Eastern India. D6 has been reported as a rare subgenotype from Indonesia, Kenya, Russia and Baltic region. D7 is the main genotype in Morocco and Tunisia. D8 and D9 are recently described subgenotypes and reported from Niger and India, respectively. Subgenotypes of genotype D may have clinical and/or viral differences. More subgenotype studies are required to conclude on subgenotype and its clinical/viral characteristics.

P-0395

An engrossing case of delta hepatitis**Ilker Sen¹, Kamuran Turker², Canan Alkim¹**

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Introduction: More than 10 million people around the world are known to be infected by the hepatitis delta, prevalence varies from region to region. Simultaneous presence of HBV is essential for the completeness of HDV life circle. In Turkey, prevalence rises towards the southeast Anatolia and Eastern Anatolia regions. Here we present a case with previously documented AntiHbs positivity who later developed delta hepatitis infection.

Case: A 67 year-old female patient, HbsAg positivity detected in 1982 before cesarean section. In april 2015 admitted with AntiHbc IgG positivity and detected for delta hepatitis, results were as follows; Hbs Ag(-), AntiHbs(-), HBV DNA(-), AntiHDV(+) and HDV RNA: 3518 copy/ml, AST: 17 U/L, ALT: 17 U/L. As we reviewed records of the previous admissions of the patient to other departments in our hospital, found out followings; in 2009, AntiHbs: 102 mIU/ml and HbsAg(-), AntiHbcIgG(+). Liver biopsy was performed and histologic activity index was found as 6/18 and fibrosis as 1/6, according to modified Knodell score system. Also in patient's history there were blood transfusions 1 month ago during cholecystectomy and several surgeries since 1980.

Discussion: Should we always trust AntiHBs formation which means to end of hepatitis B infection? When we should not? In which patient

follow-up strategy should be different after natural Anti-Hbs sero-conversion? We aimed to share this case with interesting course.

P-0396

Neglected Significant fibrosis in young HBV patients: evidence from china registry of hepatitis B

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Background and Aims: Active antiviral treatment is recommended for patients with chronic hepatitis B over 30 years of age. Younger patients in the immune-tolerant phase have minimal fibrosis. However, the distribution of fibrosis stage in non immune-tolerant patients under 30 years of age still remain unclear.

Methods and Materials: Patients aged 20–49 were selected from a nationwide hospital-based registry system (China Registry of Hepatitis B, CR-HepB). After excluding immune-tolerant patients with age 20–39, the frequency and stage of fibrosis among the non-tolerant patients were evaluated by liver biopsy and non-invasive approaches.

Results: A total of 12165 registered patients were enrolled. Of 515 with liver biopsy data, 46.7 % (57/122) were diagnosed with significant fibrosis between the age of 20 and 29 (Metavir fibrosis stage \geq F2). A comparable frequency of significant fibrosis was observed in patients aged between 30–39 [54.4 % (118/217)] and 40–49 years [59.1 % (104/176)]. This high percentage of significant fibrosis in 20–29 years patients were also validated by Fibroscan and APRI. Both liver biopsy and non-invasive methods indicated nearly 50 % of patients aged 20–29 years had significant fibrosis. In this cohort higher AST (OR = 2.57, 95 % CI 1.48–4.43) was an independent risk factor for significant fibrosis. The optimal AST cut-off value, 32.0 U/L, predicted significant fibrosis sensitively and specifically.

Conclusion: HBV-related liver fibrosis in young patients might be underappreciated. Active anti-viral treatment should be recommended to non immune-tolerant patients aged 20-29 years.

P-0397

Antifibrotic effect of oral NUCs in chronic hepatitis B infection assessed by transient elastography**Angelo B Lozada¹, Seung K Yoon², Hae L Lee², Hyun Yang², Soonkyu Lee², Si H Bae²**¹Section of Gastroenterology, Department of Internal Medicine, Makati Medical Center, Manila, Philippines; ²Division of Hepatology, Department of Internal Medicine, College of Medicine, The Catholic University of Korea, Seoul, South Korea**Background/Aims:** Liver biopsy is considered the most accurate method to evaluate current liver histology. However, recommendation of repeated biopsy for evaluation of antiviral efficacy in patients on antiviral therapy is very limited. Thus, we investigated the usefulness of Transient Elastography (TE, Fibroscan[®]) to evaluate the antiviral effect of oral nucleos(t)ides (NUCs) in chronic HBV patients.**Methods:** Patients with chronic hepatitis B on oral nucleos(t)ides, were enrolled from 2008 to 2015. Liver stiffness measurements were taken at the start of treatment and on consecutive follow ups. The relationship between the histological fibrotic score (Knodell score) and liver stiffness measurements (LSM) by TE were also analyzed. Also, we compared the changes of liver stiffness measuring LSM at the beginning and after antiviral therapy in patients with chronic HBV infection.**Results:** A total of 147 patients with chronic hepatitis B were analyzed. The most commonly used NUCs agent was Entecavir at 71 % of cases. The average score of the first LSM was 11 ± 9.16 kPa and that of follow-up LSM was 6.25 ± 2.70 kPa.**Conclusion:** Long term therapy with oral NUCs was effective in significant improvement of liver fibrosis. Transient elastography may be a useful tool for the monitoring of CHB patients on oral NUCs.

P-0398

Determinants for persistent fibrosis during nucleoside analogue therapy in chronic hepatitis B**Wai Kay Walter Seto, Ka-Shing Cheung, Kevin SH Liu, James Fung, Danny Ka-Ho Wong, Wai K Leung, Ching-Lung Lai, Man-Fung Yuen**

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Background: Changes in liver stiffness measurements (LSM) during long-term nucleoside analogue therapy in chronic hepatitis B (CHB) have not been well-investigated.**Methods:** We recruited CHB patients on long-term nucleoside analogue therapy with persistent virologic suppression (HBV DNA < 20 IU/mL on 3 occasions of at least 6 months apart) and with previous LSM indicating significant liver fibrosis, as defined by EASL-ALEH Guidelines (>9.0 kPa for normal alanine aminotransferase [ALT] and >12.0 kPa for ALT > 1–5 × upper limit of normal). Assessment included anthropometric measurements, HBV virology, and reassessment transient elastography and controlled attenuation parameter (CAP) measurements by FibroScan (Echosens, Paris, France). Hepatic steatosis was defined as CAP ≥ 222 dB/m.**Results:** In this interim analysis, 119 CHB patients (77.3 % male) were recruited. Mean age at reassessment and mean duration of nucleoside analogue therapy was 56.1 (±10.4) years and 8.5 (±3.1) years, respectively. 38 patients (31.9 %) had persistent liver fibrosis. Patients with persistent liver fibrosis, when compared to patients with fibrosis reversal, had a significantly higher mean body-mass index(26.0 and 23.6 kg/m², respectively, $p = 0.040$), mean systolic blood pressure (144 and 136 mmHg, respectively, $p = 0.029$) and mean diastolic blood pressure (82 and 77 mmHg, respectively, $p = 0.046$). There was no significant difference in the presence of hepatic steatosis among the two groups (65.8 % and 55.6 % respectively, $p = 0.290$).**Conclusion:** Metabolic parameters, including body-mass index and blood pressure, could influence fibrosis reversibility during long-term nucleoside analogue therapy. Recruitment is ongoing and the influence of other metabolic parameters (e.g. metabolic syndrome) will be analyzed.

P-0399

Assessment of response by elastography during the tenofovir treatment in hepatitis B patients**Taeheon Lee¹, Byung Ik Kim¹, Yong Kyun Cho¹, Woo Kyu Jeon¹, Hong Joo Kim¹, Mi Sung Kim², Heon-Ju Kwon²**¹Department of Internal Medicine, Kangbuk Samsung Hospital, Sungkyunkwan University School of Medicine, Seoul, Korea; ²Radiology, Kangbuk Samsung Hospital, Sungkyunkwan University School of Medicine, Seoul, KoreaThe purpose of this study was to evaluate the liver stiffness before and during the treatment and assess the usefulness of this non-invasive modality. Fourty-eight treatment-naïve patients started treatment with tenofovir. Liver stiffness measurement by a shear wave elastography and a conventional ultrasonography was performed at baseline, after median 6 and 12 months. There were no significant difference in the degree of regression of liver stiffness after median 6 ($P = 0.29$) and 12 months ($P = 0.14$). Twenty-two (45.9 %) subjects were classified as F0–1, 3 (6.3 %) as F2, 20 (41.7 %) as F3, and 3 (6.3 %) as F4. After median 6 and 12 months later, liver stiffness value of patients recorded as F0–1 was decreased mean 0.60 kPa (SD, ± 2.38) and 0.05 kPa (SD, ± 2.17), F2 as 0.81 kPa (SD, ± 1.69) and 0.99 kPa (SD, ± 3.01), F3 as 0.81 kPa (SD, ± 3.75) and 1.02 kPa (SD, ± 5.67) and F4 as 13.23 kPa (SD, ± 7.59) and 4.50 kPa (P for trend = 0.01 and 0.04 after 6 and 12 months). Liver stiffness was not significantly improved during the tenofovir treatment in patients with chronic hepatitis B during 6 and 12 months. But these result showed a trend that in a patient with advanced fibrosis on baseline stiffness stage was more improved compared to that of mild fibrosis during 6 and 12 months. Long term prospective studies are required to investigate the usefulness of elastography for relationship between elastography stiffness and treatment response in chronic hepatitis B patients treated with tenofovir.

P-0400

Dynamic changes of liver stiffness to define reverse of HBV-related fibrosis after antiviral therapy**Hong You¹, Xiaoning Wu¹, Yuanyuan Kong¹, Bo Feng², Yimin Mao³, Jiyao Wang⁴, Lungen Lu⁵, Jilin Cheng⁶, Yongpeng Chen⁷, Jihong Sun⁸, Peng Hu⁸, Xue-en Liu⁹, Xiaojuan Ou¹, Dongyang Sun¹, Jialing Zhou¹, Jidong Jia¹**¹Liver Research Center, Beijing Friendship Hospital, Capital Medical University, Beijing, China; ²Peking University People's Hospital, Beijing, China; ³Shanghai Renji Hospital, Jiao Tong University School of Medicine, Shanghai, China; ⁴Shanghai Zhongshan

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Aim: Liver stiffness values usually decreased after anti-HBV treatment in hepatitis B patients. However, the dynamic changes of stiffness to reflect fibrosis reverse were still unknown.

Methods: Chronic hepatitis B patients with anti-HBV therapy indications were treated with entecavir for 78 weeks. Liver biopsies were taken before and after treatment. HBV-DNA, liver function tests including ALT, AST, bilirubin and transient liver stiffness were detected before and every 26 weeks after treatment.

Results: A total of 390 chronic hepatitis B patients were enrolled in the study. After 78 weeks entecavir treatment liver fibrosis was reversed (Metavir score decreased ≥ 1 unit) in 58.2 % patients. Dynamic changes of liver stiffness showed the two-phase decrease manner. Fast-decreasing (mainly inflammation regression) was seen after 26 weeks treatment. In reversed group liver stiffness value decreased from 11.6 to 7.8 Kpa ($\Delta = 33$ %), whereas non-reverse group was 11.4 to 9.4 Kpa ($\Delta = 18$ %), respectively. Slow-decreasing (fibrosis regression) was from 26 to 78 weeks in reversed group of 7.2 and 5.9 Kpa at week 52 and week 78, non-reverse group was 8.6 and 8.6 Kpa, respectively. Among the factors associated with fibrosis reverse, patients' age, HBV-DNA undetectable rate, biopsy inflammation score, and changes of stiffness value were associated. Finally, dynamic changes of pathological reverse = $0.57 \times [\text{Stiffness Value (52 w-baseline)}] - 1.73$.

Conclusion: Dynamic changes of liver stiffness could be used to define HBV-related fibrosis reverse after antiviral treatment. The stiffness values of reversed patients approximately decreased by 30 % after 26 weeks and 50 % at week 78.

P-0401

Assessing fibrosis regression in treated Hep B using transient elastography and LiFA-HBV score

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Introduction/aim: The role of transient elastography (TE) to assess fibrosis regression in chronic hepatitis B (CHB) remains controversial as fibrosis grade may be overestimated in elevated ALT. Accuracy of TE may be improved with the novel LiFA-HBV score which adjusts for ALT. We studied the rate of fibrosis regression between treated and untreated CHB patients and the effect of LiFA-HBV correction.

Methods: Retrospective case-control study of treated and untreated CHB patients with baseline and repeat post-treatment TE. Primary outcome was fibrosis regression, defined as reduction by ≥ 1 grade on repeat TE. Fibrosis grade was determined by liver stiffness measurement (LSM)—F0: LSM < 5 kPa, F1: LSM 5.1–7 kPa, F2: LSM 7.1–9 kPa, F3: LSM 9.1–12 kPa, F4: LSM > 12 kPa. LiFA-HBV score used to correct fibrosis grade for ALT.

Results: 157 CHB patients with baseline LSM divided into treated cases and untreated controls. Mean age: 55 ± 12.1 years, 68 % males. 44 (28 %) received CHB treatment (20 Entecavir; 17 Lamivudine; 3 Adefovir; 4 Peg-Interferon) after baseline LSM. Distribution of advanced fibrosis ($F \geq 3$) similar between cases and controls (39 vs. 27 %, $p = \text{NS}$). Correction with LiFA-HBV

downstaged baseline fibrosis grade in 11/44 (25 %). Median duration between baseline and repeat LSM similar in cases and controls (20.7 vs. 20.3 months, $p = \text{NS}$). Fibrosis regression was observed in 18/44 (40.9 %) of treated and 36/113 (31.9 %) untreated patients, $p = \text{NS}$. There was no significant effect of LiFA-HBV correction on fibrosis regression rate between cases and controls.

Conclusion: We found no significant increase in fibrosis regression on TE between treated and untreated CHB patients over a median follow-up of 20 months. Adjustment for elevated ALT using LiFA-HBV score did not have any effect on the regression rate.

P-0402

Liver stiffness measured by transient elastography is superior to APRI and FIB-4 in CHB patients

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Aim: This study aimed to compare the accuracy of liver stiffness (LS) measured by Transient Elastography (FibroTouch) with aspartate aminotransferase to platelet ratio index (APRI) and fibrosis index based on the four factors (FIB-4) score in chronic hepatitis B (CHB) patients.

Method: A total of 106 CHB patients were performed with liver biopsy, then individual LS was measured by FibroTouch (HISKEY Medical Technologies Co., China). APRI and FIB-4 score were calculated. The correlation between each of them and pathological staging was analyzed using Spearman's rank test and compared by receiver operating characteristic (ROC) curves.

Results: There was higher correlation of LS between FibroTouch and pathological staging ($r = 0.777, P < 0.01$), compared with APRI ($r = 0.361, P < 0.01$) or FIB-4 ($r = 0.431, P < 0.01$). The best cut-off values of LS for hepatic fibrosis $F \geq 2$, $F \geq 3$ and $F = 4$ were 7.45 kPa (area under ROC curves [AUROC] = 0.902, sensitivity = 0.852, specificity = 0.827), 10.55 kPa (AUROC = 0.955, sensitivity = 0.960, specificity = 0.914) and 12.40 kPa (AUROC = 0.971, sensitivity = 1.000, specificity = 0.899), respectively. The corresponding AUROCs measured by APRI was 0.684, 0.743 and 0.679 and that by FIB-4 was 0.718, 0.790 and 0.753.

Conclusion: FibroTouch is a fairly reliable, sensitive, specific and completely noninvasive approach to assess liver stiffness in CHB patients, which is superior to APRI and FIB-4.

P-0403

Retrospective analysis of CHB patients referred to an Ontario based FibroScan® referral program

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Background and aims: The aim of this analysis is to assess the current use of FibroScan® technology by physicians in Ontario, & its role in long-term management in chronic HBV.

Methods: 3399 patients were referred to The Toronto Liver Centre for fibroscan testing from 2010 to 2014. Referrals were assessed for: patient demographics, relevant blood work (LFTs, platelets, HBV

DNA) available upon examination, & stages at which patients were referred for testing.

Results: The median age was 53 years; women were older, median of 54 vs. 52 years for men. 90.3 % of patients had a liver fibrosis staging of \leq F2 with a mean liver stiffness & SD of 5.65 kPa, 7.34, respectively. Of the 3399 patients referred, 78 % had results for ALT, 77 % for platelets, 12 % for HBV DNA; none had HBeAg/Ab results available at time of testing. Only 0.03 % (111) of patients referred with \leq F2 were recommended for therapy, based solely on family history, blood work, or viral load. Patients who had fibrosis $>$ F2 & were recommended for treatment, had a median platelet count of 101, $P = 0.0005$.

Metavir Score	HBV
Sample Size	3399
F0	
Count	920
Column %	27.10%
F0-F1	
Count	1222
Column %	36.00%
F1	
Count	505
Column %	14.90%
F1-F2	
Count	155
Column %	4.60%
F2	
Count	262
Column %	7.70%
F2-F3	
Count	72
Column %	2.10%
F3	
Count	76
Column %	2.20%
F3-F4	
Count	73
Column %	2.10%
F4	
Count	24
Column %	0.70%
Established cirrhosis	
Count	90
Column %	2.60%

Conclusion: Current CASL guidelines for the management of hepatitis B suggest HBV treatment to commence at \geq F2; with the understanding that each patient requires assessment on individual basis. FibroScan® technology provides clinicians in Ontario with an

excellent non-invasive screening tool for liver staging, by which patients can be monitored over time.

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P-0404

Correlational studies of Fibrotouch, Fibroscan and Ishak Score in patients with chronic hepatitis B

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Background and aims: This study aims to evaluate the correlation of transient elastography FibroTouch, FibroScan and Ishak Score of liver fibrosis in patients with chronic hepatitis B.

Methods: 162 patients underwent liver biopsy. Liver stiffness measurement (LSM) using FibroTouch and FibroScan were obtained in these patients. The Ishak scoring system was used for pathological staging of liver fibrosis. ALT to platelet ratio index (APRI) and FIB-4 score were calculated. The correlation of LSM, APRI, FIB-4 and pathological staging were analyzed.

Results: LSM values detected by FibroTouch and FibroScan were 16.18 ± 9.87 kPa and 15.74 ± 10.35 kPa, respectively. Pearson correlation analysis indicated that they had significant correlation ($r = 0.964$). Spearman rank correlation analysis indicated that there was significant positive correlation between FibroTouch-LSM (FT-LSM) and pathological staging ($r = 0.750$), FibroScan-LSM (FS-LSM) and pathological staging ($r = 0.789$), and low positive correlation between APRI and pathological staging ($r = 0.150$), FIB-4 and pathological staging ($r = 0.189$). The area under receiver operating characteristics (ROC) curves (AUCs) of FT-LSM for liver fibrosis $F \geq 3$, $F \geq 4$, $F \geq 5$ and $F = 6$ were 0.934, 0.880, 0.861 and 0.849, respectively; AUCs of FS-LSM were 0.948, 0.904, 0.881 and 0.869, respectively; AUCs of both LSM were significantly higher than that of APRI (0.589, 0.622, 0.596 and 0.574, respectively) and FIB-4 (0.609, 0.616, 0.574 and 0.594, respectively).

Conclusions: Both FibroTouch and FibroScan are convenient and accurate noninvasive approach for diagnosis of liver fibrosis in patients with chronic hepatitis B, which are superior to APRI and FIB-4.

P-0405

Serum ACE level as a new non-invasive fibrosis marker could be possible for HBV patients

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Background and aims: The prediction of liver fibrosis in patients with hepatitis B virus (HBV) infection is very difficult. Several reports have shown that angiotensin -converting enzyme (ACE) is involved in liver fibrogenesis. Here, we aimed to identify the non-invasive predictors of liver fibrosis development in patients with

chronic hepatitis of HBV (CHB), especially focused on serum ACE level.

Methods: Eighty patients with CHB who underwent liver biopsy in our hospital between 2013 and 2015 were enrolled. We compared with histological finding and several calculated biochemical marker-based markers, such as FIB-4 index, the aspartate aminotransferase (AST)-to-platelet ratio index (APRI) and Fibroindex along with serum ACE level. A total of 65 patients (42 male and 23 female) with chronic HBV infection except for 15 patients with fatty liver and habitual alcoholic drinking, were analyzed.

Results: Patients with an advanced fibrosis stages (F2–4) had significantly higher serum ACE levels as compared with early stages (F0–1). To identify the advanced stages, 12.8 U/L cut-off value of ACE exerted 81.0 % sensitivity, 93.8 % specificity, respectively. And, receiver-operating characteristic curves showed that an area under the curve (AUC) is 0.93. Serum ACE level revealed the best AUC value than that of any other conventional fibrosis marker and calculated biochemical marker-based markers.

Conclusion: Serum ACE level could be a novel non-invasive, easy, accurate, and inexpensive fibrosis marker of advanced fibrosis stage in patients with CHB.

P-0406

Added value of new biomarkers over APRI in hepatitis B-related fibrosis

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Background: The aspartate aminotransferase-to-platelet ratio index (APRI) has the advantage of including only two inexpensive routine laboratory tests and widespread availability, but it does not directly reflect the dynamic processes of liver fibrosis. Whether the incorporation of new biomarkers into the APRI will improve the prediction of hepatitis B virus (HBV)-related fibrosis remains unknown.

Methods: We measured seven biomarkers in 209 patients infected with HBV who underwent liver biopsies, assessing the levels of haptoglobin, apolipoprotein A1, alpha2-macroglobulin, hyaluronic acid, procollagen III N-terminal peptide, matrix metalloproteinase-2, and tissue inhibitor of metalloproteinase-1.

Results: The biomarkers that most strongly predicted significant fibrosis and cirrhosis were hyaluronic acid and procollagen III N-terminal peptide and hyaluronic acid and matrix metalloproteinase-2, respectively. The APRI-adjusted odds ratio of the multimarker score-F (based on regression coefficients of significant biomarkers) for predicting significant fibrosis was 2.72 (95 % CI 1.62–4.56). The corresponding odds ratio of multimarker score-C for predicting cirrhosis was 2.47 (95 % CI 1.67–3.66). However, the addition of the two multimarker scores into the APRI did not significantly increase the area under the receiver-operating characteristic curves for significant fibrosis (APRI without vs. with multimarker score, 0.80 vs. 0.85; $p = 0.262$) or for cirrhosis (0.76 vs. 0.82; $p = 0.372$), respectively.

Conclusions: The approach of simultaneously adding several biomarkers of liver fibrosis to the APRI did not substantially improve the diagnostic accuracy in HBV-related fibrosis. These results

highlight the importance of evaluating putative biomarkers with the use of explicitly quantitative assessments to classify their added value.

P-0407

Serum ischemia modified albumin level in chronic hepatitis B and its relation with fibrosis

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Introduction: Liver biopsy is the best method for the diagnosis of chronic hepatitis B and it is necessary for detection of hepatic parenchymal damage in chronic hepatitis B (CHB) patients scheduled for treatment. There are several studies investigating various parameters as noninvasive methods of predicting hepatic parenchymal damage. Ischemia modified albumin (IMA) levels has been shown to increase in chronic liver diseases in several studies. This study was performed to evaluate any possible association between serum IMA with the histological extent of fibrosis in patients with CHB.

Materials and methods: 74 patients with chronic hepatitis B who underwent a diagnostic percutaneous liver biopsy, 25 patients with HBV induced liver cirrhosis diagnosed with either clinical and laboratory parameters or liver biopsy and 49 healthy controls were taken into the study. Participants' blood samples were taken and centrifuged. Then IMA levels were studied collectively with spectrophotometric method.

Results: The mean serum IMA levels of the patients and healthy controls were 0.330 ± 0.116 ABSU and 0.271 ± 0.70 ABSU respectively and the difference was statistically significant ($p < 0.05$). Patient group was further divided into three according to the stage of fibrosis in liver biopsies (mild, moderate and severe). Serum IMA and IMAR levels were also found to be associated with the degree of fibrosis being higher among the patients with higher fibrosis stages.

Conclusion: In conclusion, serum IMA and IMAR levels can be considered as noninvasive serum markers of the presence and stage of liver fibrosis in patients with CHB.

P-0408

A noninvasive assessment model based on Fibroscan is good at predicting liver fibrosis progression

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Background: Little data showed the value of noninvasive biomarkers in diagnosis of liver fibrosis progression in chronic hepatitis B

patients (CHB). So, the aim of this study was to develop a noninvasive diagnostic model based on Fibroscan for assessing HBV-related liver fibrosis progression.

Methods: 40 CHB patients with paired biopsies were enrolled in this study. Ishak liver fibrosis scores were assessed on biopsy specimens by 2 pathologists. Liver stiffness measurement (LSM) was performed by Fibroscan. Twenty-seven common clinical and serum markers at baseline were assessed to derive a predictive model to discriminate the progression of liver fibrosis (progression of at least 1 fibrosis point in the Ishak score). The model created was then assessed with ROC analysis.

Results: LSM scores and platelet count (PLT) at baseline were identified by multivariate logistic regression analysis as independent factors for fibrosis progression. A fibrosis progression index constructed from the above two markers (LP index model) was established. The areas under ROC curves (AUC) were 0.786 for mild fibrosis progression (Ishak progression score >1), 0.836 for significant fibrosis progression (Ishak progression score >2), respectively. At a cutoff of <0.44, the negative predictive value to exclude significant fibrosis progression was 96.7 % with a sensitivity of 83 % and a specificity of 88 %. LP index model showed a better AUC for significant fibrosis progression (AUC 0.838) compared to APRI (AUC 0.689), FIB4 (AUC 0.517) and Forns (0.539).

Conclusion: Baseline LP index model is a useful noninvasive predictor for significant liver fibrosis progression in CHB patients.

P-0409

HBsAg and cccDNA reduction in HBeAg-positive and HBeAg-negative CHB treated with peginterferon

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Aim: To investigate the reduction in serum HBsAg and intrahepatic covalently closed-circular DNA (cccDNA) levels in patients with HBeAg-positive and HBeAg-negative chronic hepatitis B (CHB) treated with 48-week peginterferon (PEG-IFN).

Method: A total of 32 patients with HBeAg-positive CHB (group 1) and 36 HBeAg-negative CHB (group 2) were enrolled. Paired liver biopsies before and after treatment were analyzed for cccDNA. Virological response (VR) was defined as HBV DNA <2000 IU/mL (group 1 and 2) and HBeAg clearance (group 1) at 48 weeks post-treatment.

Results: Baseline cccDNA and HBsAg levels were significantly higher in group 1 than group 2. Baseline HBsAg was correlated with cccDNA in group 1 ($r = 0.557$, $P = 0.001$) but not in group 2 ($r = 0.008$, $P = 0.607$). At week 48, the decline of cccDNA and HBsAg between groups was not significantly different. The reduction of HBsAg showed a positive correlation with cccDNA decline in group 1 ($r = 0.649$, $P < 0.001$) and group 2 ($r = 0.388$, $P = 0.02$). A total of 12 (37.5 %) and 12 (33.3 %) patients achieved VR in groups 1 and 2, respectively. Responders in group 1, compared with non-responders, had significant decline in cccDNA (1.7 ± 0.9 vs. 0.5 ± 1.1 copies/cEq, $P = 0.003$) and HBsAg (2.2 ± 2.0 vs. 0.5 ± 0.6 IU/mL, $P = 0.001$). In group 2, the corresponding figures were 1.5 ± 1.4 vs. 0.5 ± 1.1 copies/cEq, $P = 0.02$, and 2.2 ± 1.0 vs. 0.5 ± 0.6 IU/mL, $P < 0.001$, respectively.

Conclusion: Serum HBsAg decline was comparable between patients with HBeAg-positive and HBeAg-negative CHB treated with PEG-IFN, which was significantly correlated with reduced cccDNA. Responders had significantly decreased in HBsAg and cccDNA compared with non-responders.

P-0410

IFN and alpha mediated excision repair pathway correlates with antiviral response against HBV infection

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Background: Previous studies identified APOBEC family as base excision repair (BER) proteins with enzymatic activity through deamination of a cytidine base in DNA and/or RNA. We hypothesized the responses to IFN α treatment of chronic hepatitis B (CHB) patients are relevant to IFN-induced deaminases and BER genes.

Methods: Ten CHB patients treated with PEGylated IFN α for 48 or 96 weeks, and six CHB-treatment naive patients as controls were recruited. Response to IFN treatment was defined as HBV DNA <1000 copies/ml with hepatitis B e antigen (HBeAg) or hepatitis B s antigen seroconversion or hepatitis B s antigen (HBsAg) decline >1 log₁₀ IU/ml. Blood and liver samples were collected, and APOBEC3 and other BER genes measured by real-time PCR. The correlations between BER gene expression levels and IFN treatment responses were studied in patients, primary human hepatocytes (PHH) and terminally differentiated HepRG cells in vitro.

Results: Compared to treatment-naïve patients, APOBEC3-A, -B, -C, -DE, and -G mRNA levels were up-regulated in IFN treated subjects. APOBEC3-A was significantly increased in IFN treated responders than non-responders. In contrast, other BER genes, NEIL3 and TDG, were down-regulated in both IFN-treated patients and in IFN-treated PHH and HepRG cells. APOBEC3 and BER gene expression at treatment endpoints partially correlated with the corresponding degree of HBsAg/HBV DNA decline and DNA level.

Conclusions: Our study suggests the expression levels of editing enzymes APOBEC3-A, -C, -F, -G, and NEIL3 and TDG correlates with IFN treatment responses in CHB patients, which may serve as biomarkers for CHB disease management.

P-0411

The impact of on-treatment ALT flares on response to peginterferon alpha-2a in CHB patients

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On-treatment alanine aminotransferase (ALT) flares would happen during peginterferon (PEG-IFN) therapy for chronic hepatitis B (CHB) patients. The correlation between the ALT flare with sustained off-treatment response or adverse outcomes was not fully studied. We

retrospectively reviewed 201 hepatitis B e antigen (HBeAg)-positive and HBeAg-negative CHB patients who underwent PEG-IFN- α -2a treatment. Nineteen percent (23/118) HBeAg-positive and 14 % (12/83) HBeAg-negative CHB patients experienced ALT flare (at least 40 U/L of ALT elevation over the baseline level). Seven (6 %) HBeAg-positive CHB patients and 3 (4 %) HBeAg-negative CHB patients experienced severe ALT flare (120 U/L of ALT elevation over the baseline). By logistic regression analysis, on-treatment ALT flare did not associated with sustained off-treatment response (OR 1.32, $p = 0.602$) in HBeAg-positive patients. In HBeAg-negative CHB patients, patients with on treatment ALT flare ($n = 12$) had higher sustained off-treatment response (50 %) than those without (24 % in the 71 patients). After multivariate analysis, on-treatment ALT flare had a trend to associate with better sustained off-treatment response (OR 3.18, $p = 0.071$). When we further stratified the degree of the ALT flare, a moderate ALT flare (80 U/L of ALT elevation over the baseline) was significant for sustained off-treatment response (OR 5.28, $p = 0.033$). None patient experienced liver decompensation in the events of ALT flare. One patient whose baseline ALT was 207 U/L, had hyperbilirubinemia during the treatment, but the case did not accompany with ALT flare. In conclusion, on-treatment ALT flare would not induce liver decompensation, and has different clinical impact on HBeAg-positive and HBeAg-negative CHB patients during PEG-IFN treatment.

P-0412

Quantification of cccDNA and pgRNA in patients with HBeAg-negative CHB treated with PEG-IFN

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Background: Covalently closed circular DNA (cccDNA) is the template for hepatitis B virus (HBV) replication via pre-genomic RNA (pgRNA) transcription. This study aimed to determine intrahepatic cccDNA and pgRNA levels in patients with HBeAg-negative chronic hepatitis B (CHB) treated with pegylated-interferon (PEG-IFN).

Method: Thirty-six patients with HBeAg-negative CHB treated with 48-week PEG-IFN and followed-up for additional 48 weeks were enrolled. HBV genotype was identified by PCR and direct sequencing methods. Paired liver biopsies from before and after treatment were analyzed for cccDNA and pgRNA by real-time PCR. Serum quantitative HBsAg was measured by a commercially available assay.

Results: The distribution of HBV genotypes B and C were 33.3 and 66.7 %, respectively. At the end of follow-up, 12 (33.3 %) patients achieved virological response (HBV DNA <2000 IU/ml). Overall, cccDNA levels at the end of treatment were significantly lower than the baseline levels (-0.5 ± 1.2 vs 0.3 ± 1.0 copies/cEq, $P < 0.001$), but pgRNA levels were not significantly changed (1.2 ± 1.4 vs 1.3 ± 2.3 copies/cEq, $P = 0.645$). Mean cccDNA was significantly decreased in responders compared with non-responders (1.5 ± 1.4 vs 0.5 ± 1.1 copies/cEq, $P = 0.022$). However, mean pgRNA reduction in responders was not different compared with non-responders (0.7 ± 1.4 vs -0.1 ± 1.9 copies/cEq, $P = 0.202$). Baseline serum HBsAg was not correlated with cccDNA and pgRNA levels. Changes in serum HBsAg levels were positively correlated with the reduction of cccDNA levels ($r = 0.364$, $P = 0.029$), but were not correlated with changes in pgRNA levels ($r = 0.308$, $P = 0.068$).

Conclusion: Responders, compared with non-responders, had significant reduction of intrahepatic cccDNA, whereas the changes in pgRNA levels were relatively small after PEG-IFN therapy.

P-0413

Kinetics of serum HBcrAg during PEG-IFN therapy in patients with HBeAg-negative CHB

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Background: Serum hepatitis B core-related antigen (HBcrAg) represents a new serum marker of hepatitis B virus (HBV) infection. However, the kinetics of quantitative HBcrAg in patients with chronic hepatitis B (CHB) receiving pegylated interferon (PEG-IFN) is currently unknown.

Method: A total of 121 patients with HBeAg-negative CHB treated with PEG-IFN for 48 weeks were enrolled. Virological response (VR) was defined as HBV DNA <2000 IU/mL at 48 weeks post treatment. Baseline liver biopsies were analyzed for intrahepatic covalently closed circular DNA (cccDNA) by real-time PCR. HBcrAg levels were assessed at weeks 0, 72 and 96 by automated chemiluminescent immunoassays.

Results: VR was achieved in 48 (39.7 %) patients. Baseline HBcrAg levels were positively correlated with cccDNA ($r = 0.380$, $P = 0.001$). Responders and non-responders had comparable baseline HBcrAg (4.2 ± 1.3 vs. 4.3 ± 1.2 \log_{10} U/mL, $P = 0.782$), but responders showed more decline of the antigen levels during and after therapy. At the end of therapy, the mean decline of HBcrAg levels from baseline in responders and non-responders were 1.1 ± 0.9 and 0.8 ± 0.8 \log_{10} U/mL, respectively ($P = 0.018$). The mean decline of HBcrAg levels in the corresponding groups at the end of follow-up were 1.2 ± 1.0 and 0.1 ± 1.2 \log_{10} U/mL, respectively ($P < 0.001$).

Conclusion: Baseline quantitative HBcrAg had positive correlation with intrahepatic cccDNA in patients with HBeAg-negative CHB. Monitoring HBcrAg levels may help predicting treatment response in patients receiving PEG-IFN therapy.

P-0414

Chronic hepatitis B treatment; real life data, an experience of a centre

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Chronic hepatitis B virus (HBV) infection is still one of the major public health problem in the World. Although, we have some choices for treatment, HBV cannot be completely eradicated with currently

available drugs. At the present time, the endpoints of chronic hepatitis treatment are suppression of HBV DNA, seroconversion of HBeAg, loss of HBsAg, improvement of liver histopathology. Real-life studies may provide better information about the efficacy and safety of drugs used in treatment. In this study, we tried to present our real life data regarding the treatment of patients with chronic hepatitis B by currently available drugs. **Materials and methods:** Patients who were diagnosed and treated at least for 1 year for chronic hepatitis B included in the study retrospectively. Patients with delta hepatitis or other co-infections, or pregnant women or could not be followed up were excluded from the study. The diagnosis of chronic HBV infection and decision for treatment was made from its biochemical, virological and histological features according to the current guidelines. Detectable HBV DNA levels after 6 months of therapy with antiviral drugs or above 2000 IU/mL with PEG-IFN alpha is accepted treatment failure. The age, gender, HBeAg positivity, HBV DNA levels, ALT levels, duration and side effects of therapy, treatment change and reasons for this were recorded.

Results: Our result were summarized in Table 1

Conclusion: Higher HBeAg seroconversion rate and HBsAg loss was achieved with PEG-IFN but side effects were also higher. It is needed to new drugs for treatment of hepatitis B.

Table 1. The Real Life Data of the Patients Treated For Chronic Hepatitis B

Features	Drugs				
	PEG-IFN	Lamivudine	Telbivudine	Entecavir	Tenofovir
Patient number	42	114	25	35	124
Female/Male	15/27	67/47	15/10	12/35	65/59
Mean age (Range) yrs.	36.7 (16-55)	42.5 (17-67)	39.4 (22-66)	39.5 (18-54)	40.2 (16-70)
HBeAg positivity	23/42	7/114	2/25	8/35	40/124
Mean treatment duration (Range)	41.8 weeks (8-48)	2.7 yrs. (1-7)	2.6 yrs. (1-4.5)	2.4 yrs. (1-6)	2.8 yrs. (1-6)
Changing due to side effects	5/42 (12%)	2/114 (1.7%)	2/25 (8%)	4/35 (11.4%)	1/124 (0.8%)
Anti HBe seroconversion	7/23 (30%)	2/7	0/2	0/8	2/40 (5%)
HBsAg loss	3/42 (7%)	1/114 (0.8%)	0/25	0/35	0/124
Changing due to treatment failure	5/42 (12%)	28/114 (24.4%)	2/25 (8%)	4/35 (11.4%)	0%134 (0%)
Relapse:15/32					
Anti HBs positivity	2/42 (4.8%)	1 (0.8%)	0/25 (0%)	0/35 (0%)	0/124 (0%)

P-0415

Placebo-controlled trial of antibody to connective tissue growth factor (CTGF) in HBV liver fibrosis

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Introduction: CTGF is a matricellular glycoprotein and central mediator of tissue remodeling and fibrosis. CTGF levels are elevated in viral hepatitis and NASH liver tissue. Inhibition of CTGF-expression prevents liver fibrosis in mouse models. FG-3019 is a human mAb to CTGF that is being developed to treat advanced liver fibrosis.

Methods: 114 subjects with chronic hepatitis B (HBV) in Asia, naïve to antiviral therapy with Ishak ≥ 2 (amended to ≥ 3), were randomized 2:1 to entecavir+FG-3019 (15 and 45 mg/kg IV, Q3 W, for 45 weeks) or entecavir+placebo. The primary endpoint was the proportion of

subjects with ≥ 1 point improvement in Ishak fibrosis score at 48 weeks (response rate) versus baseline. ClinicalTrials.gov: NCT01217632.

Results: Although the study is closed, data are not yet unblinded. FG-3019/placebo with entecavir showed excellent safety and tolerability at both dose levels: Neither local nor systemic allergic reactions were reported. At end of treatment, $>95\%$ of subjects had undetectable viral load, and the majority normalized ALTs. No abnormal laboratory trends and no evidence of liver decompensation were noted. Ishak score change is available in 76 subjects: 21 had decrease of 1 point, 18 of 2 points, 5 of ≥ 3 points, 26 were stable and 6 had increased score for an aggregate response rate of 57.9%. Unblinded data will be presented at a subsequent congress.

Conclusions: FG-3019+ entecavir was well-tolerated in subjects with advanced HBV fibrosis and FG-3019 did not appear to impair entecavir's antiviral efficacy.

P-0416

Lamivudine resistance mutations in Indonesian chronic hepatitis B patients

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Background: Lamivudine has been widely used in Indonesia for chronic hepatitis B treatment. Report from other countries showed rL80V/I, rL180M and M204V/I were the more frequent mutations found in Lamivudine resistant, in which rL180M and M204V were the most prevalent. rL80I was predominant compared to rL80 V and found more frequent in conjunction with rM204I. To date, only very limited study on Lamivudine therapy is available; hence this study was to identify hepatitis B virus (HBV) mutations associated with Lamivudine resistance in Indonesia.

Method: Eleven naïve chronic hepatitis B patients were recruited and given Lamivudine. Three patients were excluded from the analysis due to treatment withdrawal before 12 months. HBV viral load and HBeAg seroconversion were observed. Direct sequencing was used to determine HBV reverse transcriptase (RT) sequence.

Results: Eight patients were followed up for more than 12 months (average 27.1 ± 8.1 months). Seven patients (87.5%) were HBeAg(-). Three of them had viral break through after 19, 33 and 34 months of therapy. In two patients, amino acid substitutions rL80V and rM204I were found in both and L90I was found in only one. RtV207M was found in one other patient, and in addition an insertion of a single nucleotide at 1839 was found in the core region.

Conclusions: Lamivudine resistance was detected in Indonesian chronic HBV patients. In this study, mutations at rL80 V and rM204I might be a unique pattern for HBV Lamivudine resistance in Indonesian patients and can be used for better management of hepatitis B disease.

P-0417

Comparative study of efficacy of lamivudine and tenofovir in treatment for chronic hepatitis B

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Background: Chronic hepatitis B is a major global health problem. Approximately 240 million people are chronically infected with hepatitis B virus.

Methods: 60 adult HBsAg-positive patients with CHB were included. HBV genotype was identified in part of patients. Treatment effectiveness was assessed at 6 and 24 month.

Results: HBV genotype/subtype was identified in 41/60 patients. 83 % of patients had D genotype, 17 % had a genotype. Study subgroups were formed according to treatment regimen. Treatment efficiency was compared between lamivudine and tenofovir groups. There was no tenofovir superiority over lamivudine in probability of ALT normalization at 6 months (hazard ratio, 0.47; 95 % CI 0.22–1.0; $P = 0.01$). Significant difference was found in probability of aviremia at 24 months of therapy (hazard ratio 2.09; 95 % CI 1.03–4.24; $P = 0.03$).

Conclusions: Genotype D was found to be the most common in this study. Tenofovir was superior to lamivudine.

P-0418

The results of long term lamivudine therapy in anti-HBe positive chronic hepatitis B patients

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Aim: Anti-HBe positive chronic hepatitis B patients who received lamivudine therapy, were investigated for long term drug resistance and therapeutic response.

Method: Sixty one nucleos(t)ide-naive and eight cirrhotic patients receiving lamivudine, in whom the therapies were initiated between the years of 2000–2010, were enrolled. Patients with co-morbidities were excluded from the study. Predictive factors for developing resistance (gender, age, prior interferon therapy, initial ALT and HBV DNA levels, and HBV DNA positivity on sixth month) were investigated by log rank test.

Results: Lamivudine resistance were detected in 34 (49.2 %) patients during follow up period [mean: 56.2 ± 32.6 months (min: 12, max: 130)]. Initial HBV DNA and ALT levels were $101\,906\,881 \pm 191\,122\,767$ copies/mL and 134 ± 78 U/L, respectively. HBV DNA positivity on sixth month was the only significant factor for predicting resistance ($p : 0.007$). The estimated resistance rate was found % 75 for patients who had detectable HBV DNA on the sixth month of therapy, and 54 % for the others. One patient developed hepatocellular cancer (HCC).

Conclusion: Emergence of lamivudine resistance were substantially high. The sixth month HBV DNA positivity was the only reliable factor for predicting resistance.

P-0419

Cost-effectiveness of rescue therapies for LAM-resistant Patients with HBeAg-positive CHB

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Background/aim: In Chinese clinical practices, various rescue treatments are applied for lamivudine (LAM)-resistant chronic hepatitis B (CHB) patients, but the most cost-effective strategy of these treatments is unclear. This study evaluates the cost-effectiveness of five rescue strategies for LAM-resistant patients with HBeAg-positive CHB, from the perspective of short-term and long-term treatment.

Methods: Decision tree and Markov model were conducted to simulate short-term and long-term costs and effectiveness associated with five rescue therapies, respectively. Two third-line rescue therapies (base-case: Entecavir [ETV]+adefovir [ADV]; scenario: ETV+tenofovir [TDF]) were separately administrated for patients with second-line therapies failure. One-way and probabilistic sensitivity analysis were used to explore the uncertainties of model.

Results: In the base-case analysis, compared with the reference (lowest cost) treatment ADV, LAM+ADV achieved lowest cost-effectiveness ratio (CER) and incremental cost-effectiveness ratio (ICER) in the short-term treatments. For long-term therapies, LAM+ADV was served as reference treatment. ADV and ETV monotherapies were dominated by LAM+ADV because of the higher costs and lower efficacy. Although TDF and ETV+ADV generated more QALYs, the ICERs of them were both higher than the willing-to-pay threshold of \$22,833 US dollars (USD) per QALY gained (branded drugs: \$29,590 and \$76,340 USD/QALY; generic drugs: \$61,040 and \$55,210 USD/QALY). In an alternative scenario analysis, TDF would be the preferable treatment, under branded drugs price in long-term treatment.

Conclusions: For LAM-resistant patients with HBeAg-positive CHB in China, LAM+ADV is a cost-effective rescue therapy for both short-term and long-term treatment. TDF would be the preferable option when patients used potent third-line rescue strategy.

P-0420

Efficacy comparison of tenofovir and entecavir in HBeAg-positive CHB patients with high HBV DNA

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Background: Entecavir (ETV) and tenofovir disoproxil fumarate (TDF) are first-line antivirals for chronic hepatitis B (CHB) treatment. However, studies directly comparing their efficacy in CHB patients with high HBV-DNA are scarce.

Aim: To compare ETV and TDF effects in CHB patients with high HBV DNA. Method A total of 96 patients treated initially with tenofovir (TDF group) or entecavir (ETV group) were included in this retrospective study from 2012 to 2015. The following parameters were assessed: HBeAg and hepatitis B e antibody (anti-HBe) status, serum ALT and HBV-DNA levels at weeks 4, 12, 24, 36, 48, 60, 72 and 96; time to ALT normalization, undetectable HBV-DNA levels, and HBeAg seroconversion; total duration of follow-up. Finally, adverse reactions were analyzed between the treatment groups.

Results: The patients included 66 (69 %) and 30 (31 %) individuals treated with ETV and TDF, respectively, comprising 75 % males. They were 35.1 ± 4.5 and 33.7 ± 4.6 years old in ETV and TDF groups, respectively. At 48 weeks, the response rate in the TDF group was significantly higher than that obtained for ETV treated patients (90 vs 69.7 %, $P = 0.031$). At 72 weeks, less patients treated with ETV showed undetectable HBV-DNA levels compared with the TDF group (86.4 vs. 96.7 %), a non-statistically significant difference ($P = 0.125$). Only 1 ETV treated patient developed virological

breakthrough at 72w. No patients developed adverse reactions during the treatment.

Conclusion: ETV and TDF are comparable in efficacy and safety to suppress HBV-DNA replication in HBeAg-positive CHB patients with high HBV DNA.

P-0421

Evaluating eGFR changes in Chronic Hepatitis B patients with Adefovir Add-on Therapy

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Background: It is well known that adefovir (ADV) can impair renal function while Telbivudine (LdT) improves renal function in chronic hepatitis B (CHB) patients. However, a few studies focus on whether LdT still improves renal function when combined with ADV.

Methods: This was a retrospective study involving 124 CHB patients who were treated with LdT+ADV or Lamivudine (LAM)+ADV or entecavir (ETV)+ADV for at least 104 weeks between January 2010 and January 2014. The alterations in estimated glomerular filtration rate (eGFR) were compared between these three different combination therapies at baseline, 52 and 104 weeks.

Results: Among the three treatment groups, significant decreased in eGFR were observed in the LAM+ADV group, the ETV+ADV group showed mild decreased in eGFR and no significant change were observed in LdT+ADV group over 104 weeks. Moreover, in the LdT+ADV group, 43.8 % of patients with a baseline eGFR between 60 and 90 mL/min achieved normal eGFR over 104 weeks. In contrast, no patient with the same baseline eGFR achieved normal eGFR in the other two groups. In the LdT+ADV group, 6.7 % of patients with normal eGFR at baseline had abnormal eGFR after 104-weeks of treatment, which was much lower than in the other two groups ($P < 0.01$).

Conclusion: CHB patients treated with LdT+ADV therapy achieved greater eGFR improvement compared with LAM+ADV therapy and ETV+ADV therapy. LdT+ADV therapy is recommended in CHB patients who need Adefovir-Based Combination Therapy.

P-0422

Tenofovir monotherapy up to 96-weeks in patients with adefovir-resistant chronic hepatitis B

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Background/Aims: It is not clear whether tenofovir disoproxil fumarate (TDF) monotherapy and entecavir (ETV) combination therapy shows superior antiviral efficacy over TDF monotherapy in patients with adefovir-resistant hepatitis B virus (HBV).

Methods: In this multicenter trial, patients who had adefovir-resistant HBV with serum HBV DNA levels >60 IU/mL were randomized to receive TDF (300 mg/day) monotherapy ($n = 50$) or TDF and ETV (1 mg/day) combination therapy (TDF/ETV, $n = 52$) for 48 weeks. All who completed 48 weeks in either group received TDF monotherapy for 48 additional weeks.

Results: Baseline characteristics were comparable between groups, including HBV DNA levels (median 3.38 log₁₀ IU/mL). All patients had adefovir-resistant HBV mutations; rtA181 V/T and/or rtN236T. The proportion of patients with HBV DNA <15 IU/mL was not significantly different between the TDF–TDF and TDF/ETV–TDF groups at week 48 (62 vs 63.5 %; $P = 0.88$) and at week 96 (64 vs 63.5 %; $P = 0.96$). The mean change in HBV DNA levels from baseline was not significantly different between groups at week 48 (-3.03 vs -3.31 log₁₀ IU/mL; $P = 0.38$). Virologic breakthrough occurred in one patient on TDF–TDF and two patients on TDF/ETV–TDF over 96 weeks; all were attributed to poor drug adherence. None developed additional resistance mutations. Safety profiles were comparable in the two groups.

Conclusion: In patients with adefovir-resistant HBV, TDF monotherapy provided a virologic response comparable to that of TDF and ETV combination therapy, and was safe up to 96 weeks.

P-0423

Prolonged Tenofovir monotherapy for partial virologic response to Tenofovir in chronic hepatitis B

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Background/aim: The optimal management of chronic hepatitis B (CHB) patients exhibiting a partial virologic response (PVR) to tenofovir disoproxil fumarate (TDF) is currently not established. The aim of this study was to evaluate the efficacy of prolonged TDF monotherapy in treatment-naïve CHB patients exhibiting a PVR to TDF therapy.

Methods: This study included 117 treatment-naïve CHB patients treated with TDF for ≥ 48 weeks and who received continuous TDF monotherapy for ≥ 24 weeks. All patients were monitored at baseline and every 3 months during treatment.

Results: Twenty-three of 117 patients (19.7 %) showed PVR. The mean follow-up duration in PVR group was 79.0 weeks. The mean age was 49.2 years, and 15 patients (63.2 %) were men. Sixteen patients (69.6 %) were HBeAg-positive, and 10 patients (43.5 %) had cirrhosis. Nine of 23 patients (39.1 %) achieved a virologic response (VR, HBV DNA <20 IU/mL) during prolonged TDF monotherapy for ≥ 24 weeks (mean duration, 27.4 weeks). VR rate in HBeAg-positive patients was 38.4 % (5/13). Among 14 patients who did not achieve a VR during continuous TDF therapy, 10 patients had poor drug compliance. The cumulative probabilities of a VR at week 60 and 72 from treatment initiation in patients with PVR were 22.2 % and 31.6 %, respectively. The PVR was associated with HBV DNA levels at baseline, week 4, 12, and 24, and also with virologic breakthrough.

Conclusions: Long-term continuous TDF monotherapy with good medication compliance may be effective for achieving VR in treatment-naïve CHB patients exhibiting a PVR to TDF therapy.

P-0424

Tenofovir disoproxil fumarate is Effective and Safe for Lamivudine-resistant HBV Infection

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Background/aims: Tenofovir disoproxil fumarate (TDF) has potent antiviral efficacy and lack of resistance during long-term use in chronic hepatitis B (CHB) patients. The purpose of this study was to evaluate antiviral efficacy and safety of TDF in lamivudine-resistant CHB patients.

Methods: We performed a retrospective study of consecutive CHB patients who had detectable HBV DNA (greater than 50 IU/mL) and documented lamivudine-resistant mutations during antiviral treatment. Patients who had adefovir or entecavir-resistant HBV infection were excluded. They were treated with TDF monotherapy or combination with lamivudine more than 6 months. We analyzed virologic response (HBV DNA less than 20 IU/mL), biochemical and serologic responses to the TDF treatment and adverse events.

Results: A total of 101 CHB patients (HBeAg-positive 86 %, mean baseline HBV DNA 3.29 log₁₀ IU/ml) were enrolled. They were treated with TDF (n = 74) or combination with lamivudine (n = 27) for median duration of 20 months. The proportion of patients achieving virologic response at 6 and 12 months was 80.2, and 89.7 %, respectively. The mean change from baseline in HBV DNA was -2.05 log₁₀ IU/ml, and -2.14 log₁₀ IU/ml, respectively. Multivariate Cox regression analysis showed that baseline HBV DNA level was a significant predictive factor of virologic response at 12 months (HR = 0.645; 95 % CI 0.504–0.826; P = 0.001). Two patients (2.4 %) showed HBeAg loss, and no patient lost HBsAg during the treatment period. Serious adverse events or renal impairment was not observed.

Conclusions: TDF is safe and effective for complete viral suppression in patients with lamivudine-resistant HBV infection.

P-0425

Flares in patients treated with Tenofovir disoproxil fumarate (TDF) Plus Peginterferon (PEG)

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Background and aim: Factors that drive clinical outcomes following ALT flares are poorly characterized. Here, we evaluated clinical outcomes following ALT flares in patients enrolled in a 4-arm interventional CHB study.

Methods: 740 CHB patients without advanced liver disease received either TDF+PEG × 48 weeks (Arm A); TDF+PEG x16 weeks followed by TDF x32 weeks (Arm B); continuous TDF (Arm C); or PEG × 48 weeks (Arm D). ALT flares, defined as ALT >2× baseline and >5× ULN, occurring within the first 24 weeks of treatment were evaluated in context of subsequent clinical outcomes through Week 48.

Results: Overall, 29/172, 25/170, 3/174 and 25/163 patients, respectively, from Arms A-D met criteria for ALT flare; of those, 18, 16, 2, and 14 patients were baseline HBeAg-positive. A greater proportion of patients in Arms A, B and D who experienced ALT flares achieved subsequent HBeAg loss, HBsAg decline ≥ 1 log₁₀ IU/ml, or HBsAg loss compared to those who did not experience an ALT flare (table). Comparing two combination therapies, Arm A achieved higher rates of HBsAg loss (P = 0.016) following flares than Arm B. This is partly driven by HBeAg-negative patients in Arm A who attained disproportionately increasing rates of HBsAg loss and HBsAg decline ≥ 1 log₁₀ IU/ml which was not observed in Arm B.

Conclusion: Treatment-associated ALT flares, especially with TDF+PEG combination therapy, are associated with clinical endpoints related to HBV immune control. ALT flares were higher in the TDF + PEG x48w group and associated with the highest HBsAg loss.

Table: Proportion of Patients who Achieved Clinical Endpoints by ALT flare status (N=679)

	Arm A (N=172)		Arm B (N=170)		Arm C (N=174)		Arm D (N=163)	
	ALT flare		ALT flare		ALT flare		ALT flare	
	Yes (N=29)	No (N=143)	Yes (N=25)	No (N=145)	Yes (N=3)	No (N=171)	Yes (N=25)	No (N=138)
HBeAg loss (%) ^a	38.9	10.4	18.8	11.4	0	5.6	21.4	7.7
HBsAg decline ≥ 1log ₁₀ IU/ml (%)	34.5	11.9	28.0	4.8	0	1.2	24.0	11.6
HBeAg+ (%)	27.8	11.0	43.8	6.3	0	2.0	28.6	12.8
HBeAg- (%)	45.5	13.1	0	3.0	0	0	18.2	10.0
HBsAg loss (%)	24.1	1.7	4.0	0.7	0	0	12.0	0.4
HBeAg+ (%)	27.8	1.8	6.3	0.6	0	0	21.4	0
HBeAg- (%)	18.2	1.6	0	0.8	0	0	0	0.8

P-0426

The efficacy of Tenofovir in patients with chronic hepatitis B

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Aim: Tenofovir disoproxil fumarate (TDF) is an effective and reliable nucleotide reverse transcriptase inhibitor used in the treatment of chronic hepatitis B (CHB). The present study aimed to determine the virological response in patients with CHB who had undergone TDF therapy and had been followed up at five different centers.

Method: A hundred-eighty seven patients, who had been followed up at five different centers between 2008 and 2014, were included in the present retrospective study. ALT, AST, HBeAg, anti-HBe, HBVDNA levels were recorded by reviewing the patient files.

Findings: Of the 187 patients included in the study, 105 (56.1 %) were male; the mean age was 38 ± 14 years; 56 patients (29.9 %) were HBeAg positive, and 131 (70.1 %) were HBeAg negative. The mean AST and ALT levels were 43 ± 26 and 36 ± 18 , respectively. In 78 patients (41.7 %) the HBVDNA level was >107 before treatment. The virological response developed at the end of first year in 141 patients (75.4 %). This ratio was 87.7 % at the end of second year.

Conclusion: TDF is a potent antiviral agent in treatment of CHB.

P-0427

Comparison of the effectiveness of Tenofovir in Nucleoside analogue naive and experienced in CHB

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Objective: To compare the effectiveness and influences of tenofovir TDF monotherapy between Nucleoside analogue NA-naive and experienced in chronic hepatitis B CHB patients.

Method: A total of 101 patients who completed 72 weeks of NA naive group $n = 36$ and NA experienced group $n = 65$ both treated by TDF were retrospectively studied. The effectiveness data in the two groups were collected and analyzed.

Results: HBV DNA levels were progressively declined to the limit of HBV DNA detectable level at 24–36 week in NA naive group and in NA experienced group treated by TDF monotherapy. There was no significantly difference between them $P > 0.05$. The rates of HBV DNA <100 IU/ml were 8.3, 44.4, 72.2, 87.5, 96.3, 96.2 % in NA naive and 18.2, 39.4, 75.0, 88.1, 91.2, 91.8 % in NA-experienced treated by TDF monotherapy at 4¸12¸24¸36 ¸48¸72th week. There was no significantly difference between them in the whole time by regression analysis of Kaplan–Meier &chi = 1.128, $P = 0.288$. ALT normalization rates in NA naive and NA experienced were similar at 4¸12¸24¸36¸48¸72 week all $P > 0.05$.

Conclusion: There was no significantly difference in NA naive and in NA-experienced CHB treated by TDF monotherapy for 72 weeks.

P-0428

Two-year real life data of Tenofovir Disoproxil Fumarate to chronic hepatitis B patients in Taiwan

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Aim: The aim of this study is focus on the treatment efficacy and renal tolerability of Tenofovir Disoproxil Fumarate (TDF) to patients with chronic hepatitis B (CHB).

Method: Data from subjects of Taichung Verteran General Hospital were retrospectively collected from 2012 to 2014. The inclusion criteria included CHB, serum HBV DNA more than 2000 IU/ml and high serum alanine aminotransferase (ALT). The enrolled individuals had persist taken TDF at least 1 year.

Results: Among all 100 enrolled subjects, 28 and 24 patients had positive HBeAg and treatment-experienced respectively. The 2-year biochemical response (ALT less than 40 U/L) was 95–100 %, virologic response (HBV DNA less than 20 IU/ml) was 86 % to 98 %, and serologic response (HBeAg seroloss) was 33 %. There was no significant difference exist between HBeAg positive cases and negative ones, or treatment-naive cases and -experienced ones. Renal function of these subjects remain stable over 24 months.

Conclusion: TDF treatment is considered as a efficient and safe therapy to the individuals with CHB.

P-0429

Efficacy and safety of Tenofovir in patients with chronic hepatitis B: four year real-life data

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Background/aims: This study sought to investigate the long term real-life efficacy and safety of Tenofovir therapy in treatment-naive or experienced, HBeAg positive and negative chronic hepatitis B patients.

Materials and methods: This was a multicentered study with participation of seven centers across Turkey. A total of 206, 177, 118 and 67 patients who completed their 1st, 2nd, 3rd and 4th year of therapy, respectively, were enrolled.

Results: In 206 patients, after 4 years of treatment, 39.1 % had HBeAg loss and 19 % achieved HBeAg seroconversion while HBsAg seroconversion or loss was not observed. By the end of the 1st, 2nd, 3rd and 4th year, the HBV DNA suppression (<300 copy/mL) was 51,

65, 70 and 89 %, respectively, in HBeAg positive patients, and 84, 93, 95 and 100 % in HBeAg negative patients, respectively. The alanin aminotransferase (ALT) normalization by the end of 4th year was found to be 78.9 % in HBeAg negative patients and 65.6 % in HBeAg positive patients. After having completed their 4th year, only 1.9 % of patients had serum creatinine levels higher than 1.2 mg/dL. **Conclusion:** Tenofovir monotherapy leads to substantial HBV DNA suppression, ALT normalization and HBeAg seroconversion in patients evaluated with real-life data. Tenofovir is a well tolerated, safe antiviral agent in long term usage for renal functions.

P-0430

Cost-effectiveness of lamivudine plus adefovir combination therapy in chronic hepatitis B patients

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Background/aim: Lamivudine (LAM) plus adefovir (ADV) combination therapy is clinically efficacious for treating chronic hepatitis B (CHB) patients in China, but no pharmacoeconomic evaluations of this strategy are available. The aim of this study was to examine the cost-effectiveness of LAM plus ADV combination treatment compared with five other nucleos(t)ide analogue monotherapies [LAM, ADV, telbivudine (TBV), entecavir (ETV)].

Methods: To simulate the lifetime (40-year time span) costs and quality-adjusted life-years (QALYs) for different therapy options, a Markov model that included five initial monotherapies and LAM plus ADV combination as an initial treatment was developed. Two kinds of rescue combination strategies (base-case: LAM+ADV then ETV+ADV; alternative: direct use of ETV+ADV) were considered separately for treating patients refractory to initial therapy. One-way and probabilistic sensitivity analyses were used to explore model uncertainties.

Results: In base-case analysis, ETV had the lowest lifetime cost and served as the reference therapy. Compared to the reference, LAM, ADV and TBV had higher costs and lower efficacy, and were completely dominated by ETV. LAM plus ADV combination therapy or TDF was more efficacious than ETV, but also more expensive. Although the incremental cost-effectiveness ratios of combination therapy or TDF were both higher than the willingness-to-pay threshold of \$20,466 US dollars (USD)/QALY gained for the reference treatment, in an alternative scenario analysis LAM plus ADV combination therapy would be the preferable treatment option.

Conclusion: ETV and LAM plus ADV combination therapy are both cost-effective strategies for treating Chinese CHB patients.

P-0431

HBV relapse and HBsAg loss after LAM and ETV therapy in HBeAg-negative patients with HBsAg ≤ 200 IU/mL

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Background and aims: To investigate the incidence and predictors of HBV relapse and HBsAg loss after discontinuing lamivudine and entecavir treatment in HBeAg-negative patients with end-of-treatment HBsAg ≤ 200 IU/mL.

Methods: A total of 102 patients (27 cirrhosis at entry), who were treated with lamivudine (n = 33) or entecavir (n = 69) previously and had post-treatment follow-up for at least 12 months were recruited. All patients achieved end-of-treatment HBsAg ≤ 200 IU/mL and fulfilled the stopping criteria of the APASL 2008 or 2012.

Results: The 6-year cumulative rates of virological and clinical relapse and HBsAg loss were 34.9, 23.8 and 61.5 %, respectively. Cox regression analysis revealed that age, cirrhosis, longer treatment duration and lower end-of-treatment HBsAg levels were independent factors for virological relapse. HBV genotype C and lower end-of-treatment HBsAg levels were independent factors for HBsAg loss. The 6-year virological relapse rates in HBsAg levels <100, 100–150 and >150 IU/mL were 18.9, 46.2 and 66.9 %, respectively, and HBsAg loss rates were 71.3, 56.2 and 25 %, respectively. Furthermore, the 6-year virological relapse rates in patients with age <55 and ≥ 55 years were 19.4 and 53.2 % respectively. A combination of age (<55 years) and HBsAg level (<100 IU/mL) had the lowest virological relapse rate (6 years: 8.6 %) and age (≥ 55 years) and HBsAg level (>150 IU/mL) had the highest virological relapse rate (6 years: 77 %). None of these patients experienced hepatic decompensation off-therapy.

Conclusions: Patients with HBsAg <100 IU/mL could achieve the highest HBsAg loss and lowest HBV relapse rates off-therapy. Age (<55 years) play an important role in HBV relapse.

P-0432

Combination of LdT and ADV is efficacious and safe for CHB patients who failed LAM/ADV/LdT treatment

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Background: Aim of this study to investigate the efficacy and safety of telbivudine combined adefovir in chronic hepatitis B patients with lamivudine/telbivudine/adevovir treatment failure.

Methods: We designed an open label and retrospective clinical trial to assess the efficacy and safety of combination of telbivudine and adefovir in 106 chronic hepatitis B patients with lamivudine/telbivudine/adevovir treatment failure. Quantitative HBV DNA measurement below 100 IU/ml was used. ALT, HBV DNA and HBeAg were quantitatively measured at baseline and every 3 months. **Results:** 78 patients (68 male) with a median age of 27.3 years (range 20–62 years) were included. 60.3 % (47/78) of patients had lamivudine experience, and 39.7 % (31/78) had prior exposure to telbivudine. At baseline median ALT was 3.32 ULN (range 1.23–12.2 ULN) and HBV DNA was 6.17 log₁₀ IU/ml (range 4.7–8.1 IU/ml). Median treatment duration was 33.7 months (range 3–53 months), without significant clinical side effects. The median HBV DNA level dropped highly significant by 4.0 logs (range 2.2–6.1 log; p below 0.001) and 71.8 % (56/78) patients became HBV DNA undetectable (below 100 IU/ml). 10.1 % (10/69) of patients achieved HBeAg seroconversion on ETV therapy.

Discussion: Combination of telbivudine and adefovir therapy can significantly improve liver function and effectively decrease serum level of HBV DNA in patients with chronic hepatitis B infection.

P-0433

Efficacy of LDT+ADV combination therapy and ETV monotherapy For CHB patients with high viral load**Wei Gou**

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Background/aim: Suboptimal response and drug resistance often occur in chronic hepatitis B (CHB) patients with high baseline viral load (HBV-DNA 107 copies/mL) treated with commonly used monotherapy antiviral drugs. The aim of our study was to compare the safety and efficacy of telbivudine (LdT)+adefovir (ADV) versus monotherapy entecavir (ETV).

Methods: We randomly assigned 80 patients from Qingdao Infectious Disease Hospital from February 2011 to April 2012 to receive LdT 600 mg/daily plus ADV 10 mg/daily for 96-weeks or monotherapy ETV 0.5 mg/daily for 96-weeks. Liver and renal function, myocardial enzymes, HBV-DNA, hepatitis B e antigen (HBeAg) and drug resistance were assessed at 12, 24, 48 and 96-weeks and adverse drug reactions were observed.

Results: Liver function and virological indicators showed significant improvement at 12, 24, 48, and 96-weeks of treatment compared to baseline. At 96-weeks, ALT normalization of LdT+ADV was significantly higher than ETV ($\chi^2 = 4.501$, $P = 0.05$). HBV-DNA loss and HBeAg seroconversion in LdT+ADV at 12, 24, 48-weeks were not significantly different from ETV. At 96-weeks, HBV-DNA loss and HBeAg seroconversion of LdT+ADV were significantly higher than ETV ($\chi^2 = 5.165$ and 7.040 , $P = 0.05$). In the LdT+ADV ($N = 40$), no patient experienced virologic breakthrough, 2 patients did with ETV.

Conclusion: For CHB patients with high viral load, initial combination therapy with LdT+ADV yielded significantly improved liver function and virological indicators. Furthermore, LdT+ADV drug resistance rate was lower than ETV. A prospective study is recommended to confirm these findings.

P-0434

Clinical effects of Entecavir vs Lamivudine plus adefovir on HBV-related compensated cirrhosis

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Aims: To investigate clinical effects of two anti-viral therapies entecavir (ETV) alone vs Lamivudine plus adefovir combination (LAM + ADV) on HBV related compensated liver cirrhosis.

Patients and methods: Patients ages from 18 to 70 years old who clinically diagnosed as HBV-induced compensated cirrhosis. Patients received either entecavir alone or Lamivudine plus adefovir

combination. Data are collected at baseline and every 3 months of follow-up.

Results: Totally 621 patients (526 in ETV group vs 97 in LAM+ADV group) were included. The HBV-DNA undetectable rate were 84 and 88 % in ETV group after 12 and 24 months of therapy vs 75 and 78 % in LAM+ADV group ($P = 0.236$, 0.344). HBeAg loss rate were 17.8 and 21.7 % in ETV group after 12 and 24 months vs 28.5 % and 33.3 % in LAM+ADV group ($P = 0.406$, 0.455). Liver stiffness decreased from baseline about 25 and 35 % after 12 and 24 months of therapy in both groups. There were no significant differences between ETV group and LAM + ADV group in biochemical response, Child-Pugh score or MELD score changes after treatment of 12 and 24 months. Till now, ten patients had decompensation events (one-year and two-year incidence rate were 1.7 and 2.1 %), nineteen patients had hepatocellular cancer (one-year and two-year incidence rate were 3.5 and 7.1 %).

Conclusions: After 2 years treatment with entecavir alone or lamivudine plus adefovir combination therapy, there were no significant differences in virological, serological, biochemical response among compensated cirrhosis. Both of the treatment could achieve HBV DNA suppression in most of cirrhosis patients.

P-0435

Combination of ADV and ETV therapy is effective in CHB patients who failed to ADV monotherapy

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Objective: To evaluate the efficacy of a new therapeutic strategy of combining ADV and ETV therapy in chronic hepatitis B patients who failed to ADV monotherapy.

Methods: Patients who showed suboptimal response which was defined as that the decline of HBV DNA level was more than 1 log₁₀ copies/mL, but still detectable by real-time PCR after 48 weeks of ADV monotherapy were included. A cohort of 66 consecutive patients with chronic hepatitis B from liver clinics in the third affiliated hospital of SUN Yat-sen University was retrospectively studied. All patients started combination therapy for the following indications: AVR ($n = 23$), SOR ($n = 43$). CVS was defined as undetectable serum HBV DNA below 100 IU/mL. The serum ALT, TBIL, ALB, HBV DNA were monitored with an interval of 12 weeks.

Results: Among the patients, 32.0 % (21/66) had prior treatments, of whom 90.5 % had lamivudine treatment failure (AVR or SOR), and 68 % (45/66) were adefovir naive treatment, of whom 33 patients were SOR to adefovir, 12 patients were AVR to adefovir. After 48-week follow up, the biochemical response (BR) rate (normalization of alanine aminotransferase levels) were 34.4 % (11/32); the virological response (VR) rate (HBV DNA level below 100 IU/mL) were 71.2 % (47/66); the seroconversion rate (from HBeAg to HBeAb) were 2.4 % (1/43); the virologic breakthrough (VB) rate were 1.5 % (1/66). All patients in two groups showed good and comparably tolerant and safe data to therapies.

Conclusion: Optimized combination therapy of ADV plus ETV may be good choice for chronic hepatitis B patients who failed to ADV monotherapy.

P-0436

Telbivudin treatment experience of a single center

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Background/aims: Telbivudine is a L-nucleoside analogue with potent antiviral activity against hepatitis B virus (HBV). The aim of this study was to monitor the side effect such as myopathy by following the levels of creatinine phosphokinase, viral load and glomerular filtration rate (GFR).

Methods: 54 patients were enrolled. The level of HBV DNA, ALT, CK and creatinine were monitored at the beginning of the study and every 3 months. The patients' GFR was calculated every month.

Results: 54 patients were included in the study. 11 patients were on Lamivudine treatment before the Telbivudine treatment for an average of 23 months. The patients took Telbivudine for 34.4 months (9–50 months). HBVDNA became negative in 42 (77.7 %) patients after 6 months therapy. In 42 patients completing 12 months of therapy HBVDNA became negative in 40 (95.2 %) of them. In 2 (3.7 %) patients breakthrough was observed. This patients' baseline HBVDNA was 3.2×10^4 IU/ml. At the end of the sixth month treatment HBVDNA decreased 5.12 log, in 13(50 %) patients CK increase was observed from which 11 (20.3 %) were asymptomatic, 2 (3.7 %) had myopathy symptoms. The improvement of GFR at the end of the sixth month was $6.1 \text{ ml/min/1.73 m}^2$; ($p < 0.001$ n = 54).

Conclusion: In our study telbivudine was shown to be a potent antiviral therapy. 88.7 % of the patients HBVDNA became negative. CK level monitoring was proven to be an important biomarker in telbivudine treatment, however not an absolute criteria for treatment interruption. GFR was improved during the treatment period however needs further evaluation with larger group studies.

P-0437

Telbivudine treatment in chronic hepatitis B patients: a prospective, real world experienced study

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Background: Telbivudine has been approved for treatment of chronic hepatitis B. We conducted a real world study to evaluate the efficacy of this drug in Taiwan.

Methods: 160 intent-to-treat chronic hepatitis B patients to whom telbivudine was prescribed were included for a prospective, observational study.

Results: They were classified into 6 groups according to the clinical conditions. Group I: HBeAg-positive, treatment-naïve patients (n = 16); Group II: HBeAg-positive and previously treated by entecavir (n = 15); Group III: HBeAg-positive, HCC patients receiving chemotherapy (n = 8); Group IV: HBeAg-negative, treatment-naïve patients (n = 39); Group V: HBeAg-negative, cirrhotic patients (n = 34); and Group VI: HBeAg-negative, HCC or other cancer patients receiving chemotherapy (n = 48). The virological response rates assessed as HBV-DNA < 300 copies/mL were 50.0, 66.7 %, NA, 91.3, 57.1, and 100 % at week-104 for Group I to VI,

respectively. The accumulative virological breakthrough rates during the 2-year follow-up periods were 2/16 (12.5 %), 4/15 (26.7 %), 0/8 (0 %), 3/39 (7.7 %), 5/34 (14.7 %), and 0/48 (0 %), respectively. An increase of creatine kinase (CK) levels was found for all groups. Multivariate analysis showed that liver cirrhosis ($P = 0.008$) and body height ($P = 0.0167$) independently associated with a CK level > 300 IU/mL. A profound increase of the estimated glomerular filtration rate was found between week-48 and week-104 ($P < 0.001$). **Conclusions:** Despite effective virological suppression, virological breakthrough remained a major obstacle for the clinical use of telbivudine. Renal function improvement mainly occurred in the second year of treatment.

P-0438

Combination of LdT and ADV is efficacious and safe for CHB patients with LAM/LdT experience

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Background: Combination therapy for chronic hepatitis B infection is recommended for patients with treatment failure to earlier antiviral therapy; however, data on outcomes are limited. Aim of this study to investigate the efficacy and safety of telbivudine combined adefovir in lamivudine pretreated and telbivudine pretreated patients with chronic hepatitis B infection.

Methods We designed an open label and retrospective clinical trial to assess the efficacy and safety of combination of telbivudine and adefovir in 78 chronic hepatitis B patients. Quantitative HBV DNA measurement below 100 IU/ml was used. ALT, HBV DNA and HBeAg were quantitatively measured at baseline and every 3 months. **Results:** 78 patients (68 male) with a median age of 27.3 years (range 20–62 years) were included. 60.3 % (47/78) of patients had lamivudine experience, and 39.7 % (31/78) had prior exposure to telbivudine. At baseline median ALT was 3.32 ULN (range 1.23–12.2 ULN) and HBV DNA was 6.17 log₁₀ IU/ml (range 4.7–8.1 IU/ml). Median treatment duration was 33.7 months (range 3–53 months), without significant clinical side effects. The median HBV DNA level dropped highly significant by 4.0 logs (range 2.2–6.1 log; p below 0.001) and 71.8 % (56/78) patients became HBV DNA undetectable (below 100 IU/ml). 10.1 % (10/69) of patients achieved HBeAg seroconversion on ETV therapy.

Discussion: Combination of telbivudine and adefovir therapy can significantly improve liver function and effectively decrease serum level of HBV DNA in patients with chronic hepatitis B infection.

P-0439

Effects of ETV or TDF on renal function in patients with HBV-related cirrhosis: outcome at 2 years

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Background/aims: There is controversy about renal effects of nucleos(t)ide analogues in patients with chronic hepatitis B(CHB), especially compensated and decompensated HBV-related cirrhosis. We aimed to compare the impact on renal function in HBV-related cirrhosis patients treated with entecavir (ETV) or tenofovir (TDF).

Methods: From 2012 to 2015, 239 consecutive treatment-naïve patients with HBV-related compensated and decompensated cirrhosis treated with ETV (n = 166) and TDF (n = 73) for at least 96 weeks with baseline estimated glomerular filtration rate(eGFR) <50 mL/min, were enrolled. Serum creatinine-based equations (i.e., Modification of Diet in Renal Disease) was used to estimated GFR (eGFR).

Results: The median age of the patients (158 men, 81women) was 56.0 years. The baseline characteristics were comparable between these two groups. In ETV-treated patients, the mean eGFR decreased by 6.0 % at week 96 compared with the eGFR at baseline (MDRD formula in mL/min/1.73 m²). In TDF-treated patients, the mean eGFR decreased by 6.1 % at week 96 compared with the eGFR at baseline. A significant reduction in the eGFR was found in two groups. Similar results were shown for creatinine. By multivariate analysis, the only significant factor associated with an increase in eGFR >20 % was pre-existing renal insufficiency [adjusted odds ratio (OR), 0.809; 95 % confidence interval (CI), 0.668–0.968; P = 0.031].

Conclusions: In patients with HBV-related compensated and decompensated cirrhosis, ETV and TDF have similar renal safety profile and treatment with ETV or TDF can potentially induce renal impairment.

P-0440

Entecavir versus Tenofovir in Treatment of Nucleoside Analogue-Naive Chronic Hepatitis B Patients

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Aim: Entecavir (ETV) and tenofovir (TDF) are the two first-line therapies recommended in the treatment of chronic hepatitis B because of having high genetic barriers against resistance. We aimed to compare efficacy of these drugs and to evaluate predictors of viral suppression.

Method: This multicenter retrospective study was conducted in nucleos(t)ide analogue-naive chronic hepatitis B(CHB) patients from different 6 centers. Of the 252 patients, 166 received ETV and 86 TDF. The two groups were similar in terms of age, gender, baseline ALT levels and fibrosis scores. ETV had significantly higher baseline HBV DNA, histological activity index and a lower HbeAg seropositivity. Treatment duration was longer in ETV group (P < 0.001). In univariate analysis, undetectable HBV DNA and ALT normalization rates were detected significantly higher in ETV groups (P < 0.001 and 0.049 respectively). There was no significant difference between groups in terms of HBeAg seroconversion, virological breakthrough, time to virological breakthrough and time to ALT normalization. Entecavir was more effective in reducing HBV DNA levels at the 3rd, 6th and 12th months of the treatment (P = 0.06, 0.021 and 0.012, respectively). However, multivariate Cox regression analysis indicated that treatment with TDF compared to ETV had an increased probability of achieving complete viral suppression (HR = 1.66; 95 % CI 1.21–2.33; P = 0.010). HbsAg seroconversion was occurred in only one patient in ETV group.

Conclusion: Entecavir leads to an early response on HBV DNA decline in the first year of the treatment. However, in longterm tenofovir is 1.66 times more successful than entecavir in achieving virological suppression.

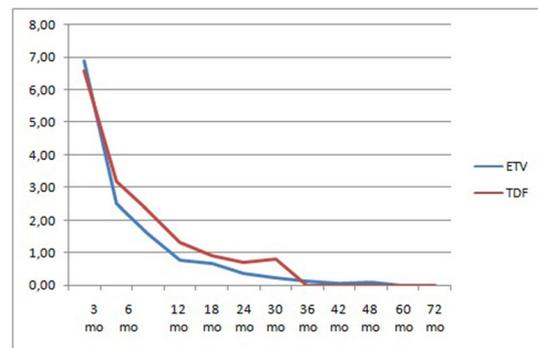


Figure 1. HBV DNA (log₁₀) decline over time, by entecavir (ETV) and tenofovir (TDF) therapy

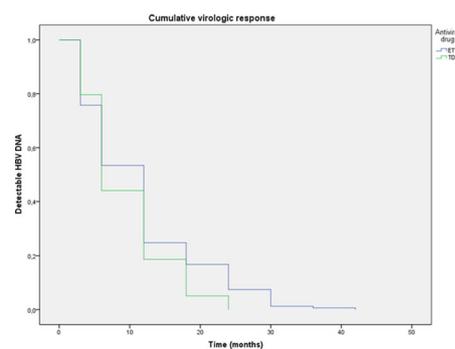


Figure 2. Cumulative probabilities of undetectable HBV DNA, by entecavir (ETV) and tenofovir (TDF) therapy (Log Rank Test, p = 0.62)

Table 1. Univariate analysis results: Comparison of entecavir and tenofovir groups in terms of baseline demographic and laboratory characteristics, and treatment response indicators

	ETV (n=166)	TDF (n=86)	P value
Age (years)	43 (18-81)	42 (18-71)	0.488
Gender male, n (%)	118 (71.0)	48 (55.8)	0.555
Weight of patient	75 (50-104)	81(47-105)	0.004
Treatment duration, months	48 (12-72)	18 (12-72)	<0.001
Baseline serum HBV DNA (log ₁₀ IU/ml)	6.92(1.41-9.48)	6.6(3.47-8.85)	0.037
Baseline serum ALT (IU/L)	89 (20-708)	92 (14-900)	0.296
Elevated ALT before therapy, n (%)	146 (88)	76 (87.1)	0.922
Liver biopsy done, n (%)	155 (93.4)	58 (67.4)	<0.001
Baseline HAI (Ishak)	9 (2-18)	8 (2-15)	0.036
Baseline fibrosis (Ishak)	2 (0-6)	2 (0-5)	0.550
HBeAg seroconversion	1	2	0.294
HBeAg seroconversion*	56 (33.7)	41 (52.3)	0.004
Time to HBeAg seroconversion, months	10 (7-6)	5 (6-5)	0.294
ALT normalization, n (%)	37 (12-120)	15 (3-24)	0.048
Time to ALT normalization, months	133 (91,7)	63 (82,9)	0.049
Undetectable HBV-DNA, n (%)	3 (3-72)	6 (3-30)	0.611
Time to undetectable HBV-DNA, months	161 (97)	59 (68,9)	<0.001
Virological breakthrough	12 (3-42)	6 (3-24)	0.321
Time to virological breakthrough	20 (12,6)	6 (10)	0.599
	24 (6.48)	18 (12-24)	0.312

*HBeAg seroconversion rates were evaluated for HBeAg positive patients.

P-0441

The reduction of HBV DNA levels at the third month for predicting virological response

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Background: We aimed to determine whether the reduction of HBV DNA at the third month of treatment is a predictor for response at the first year and also we compared the efficacy of entecavir (ETV) and tenofovir (TDF) in terms of virological, serological, biochemical responses in nucleos(t)ide-naïve CHB patients.

Materials and methods: A retrospective observational study was conducted in a single center and treatment-naïve CHB patients who received either TDF (245 mg/day) (n = 36) or ETV (0.5 mg/day) (n = 38) for at least one year were included.

Results: Both arms were similar in terms of demographic characteristics and baseline status. HBV DNA responses with ETV from first to sixth year of treatment were found to be 71, 96.6, 95.6, 100, 100, 100 % respectively. HBV DNA negativity with TDF was 69.4 % at the first year, 96.9 % at the second year, 95.8 % at the third year and was 100 % after the fourth year. No significant differences were noted between two treatment arms in terms of ALT normalization, HBeAg clearance and antiHBe seroconversion rates. Patients with lower baseline viral load ($\leq 8 \log_{10}$ IU/mL), HBeAg-negative patients and patients whom HBV DNA levels reduced more than $4 \log_{10}$ IU/mL at the third month had higher HBV DNA negativity at the end of the first year.

Conclusions: ETV and TDF were similarly effective for the treatment of CHB $\geq 4 \log_{10}$ IU/ml decline in HBV DNA levels at the third month of treatment could be considered to be an indicator for virological response at the first year.

P-0442

Comparison of entecavir and tenofovir effectiveness in treatment-naïve chronic hepatitis B patients

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Background: The hepatitis B virus (HBV) is estimated to have infected more than 2 billion people worldwide. Approximately one million people die from chronic hepatitis B (CHB)-related complications (cirrhosis, liver carcinoma) every year. Entecavir (ETV) and tenofovir (TDF) are potent antiviral agents in treatment of patients with chronic hepatitis B. We evaluated efficacy and safety of ETV and TDF therapy in treatment-naïve CHB patients.

Methods: We performed a retrospective study. 193 treatment-naïve CHB patients who were followed in the Sakarya University Education and Research Hospital-Infectious Diseases Clinic were included in this study. The patients were evaluated according to characteristics, hepatitis B e antigen (HBeAg) clearance and seroconversion in HBeAg-positive patients, HBsAg clearance and development of AntiHBs.

Results: 193 CHB patients were identified; 90 (46 %) patients were treated with ETV and 103 (53 %) patients were treated with TDF. ETV and TDF therapies were given to the patients with HBV-DNA greater than $7 \log_{10}$ IU/mL. Characteristics of these patients are presented in Table 1. HBeAg seroconversion rates in HBeAg-positive patients were 14.2 % in the ETV group and 12.1 % in the TDF group; the difference was not significant ($p > 0.05$). The mean time to HBeAg seroconversion in the ETV and TDF groups were 15 and 29 months. Six (6.6 %) patients on ETV therapy had virological breakthrough ($p = 0.009$). None of the patients had HBsAg clearance in each group.

Conclusions: ETV and TDF are effective antiviral agents for treatment-naïve CHB patients, but 6.6 % of patients on ETV therapy had virological breakthrough. Both drugs were well tolerated and renal toxicity were not observed.

Table 1: Characteristics of treatment-naïve CHB patients

Characteristics	ETV (n:90)	TDF (n:103)	p-value
Age (years), mean	47.3	43.3	0.03
Gender, male	57	62	>0.05
Treatment duration (months), mean	39.1	36.3	>0.05
HBeAg positivity	21	33	>0.05
HAI, mean	7.8	7.9	>0.05
Fibrosis, mean	2.1	2.5	>0.05

HAI: Histologic Activity Index

P-0443

Long term entecavir treatment results in viral suppression and fibrosis regression

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Background: Although entecavir (ETV) is effective and safe in patients with chronic hepatitis B (CHB), but its long term efficacy and safety need to be established.

Methods: We evaluated clinical outcome in HBeAg-positive and negative treatment-naïve HBV patients treated with ETV for more than 6 months. Fibrosis regression was estimated by liver stiffness (LS) value obtained by repeated transient elastography.

Results: A total of 855 treatment-naïve CHB patients (HBeAg positive 381 patients and HBeAg negative 474 patients) have been treated with ETV 0.5 mg up to 8 years in this study. Complete virological response, which was defined as undetectable serum HBV-DNA (< 20 IU/mL), was achieved in 62.5, 89.1, 94.6, and 98.9 % of

HBeAg-positive, and in 73.9, 91.4, 93.5 and 93.5 % of HBeAg-negative patients at 1-, 3-, 5- and 7-year, respectively. In HBeAg positive patients, HBeAg seroconversion was observed in 10.9, 33.1, 49.5 and 60.6 % of patients at 1-, 3-, 5- and 7-year. The median LSM decreased from 12.4 kPa at baseline to 9.4 kPa at 1-year, 7.1 kPa at 3-year, and 7.2 kPa at 5-year, respectively. Using cutoff value of 11 kPa for diagnosis of cirrhosis, 60.5 % patients had cirrhosis at baseline, and the proportion of cirrhosis decreased to 48.8, 34.9 and 25.6 % after 1, 3, and 5-year ETV treatment. Cumulative incidences of hepatocellular carcinoma were 1.0, 2.9, 5.4 and 8.0 %, at 1-, 3-, 5- and 7-years, respectively.

Conclusions: Long term ETV treatment showed considerably good clinical outcome including virological response and liver fibrosis regression, and a favorable safety profile for treatment-naïve CHB patients.

P-0444

HBsAg loss in patients with chronic HBV treated with Entecavir: a retrospective case series

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We retrospectively investigated our patients who have been followed up in our gastroenterology and infectious diseases clinic between January 2007 and September 2015. All the patients were followed up at least 6 months before therapy to ensure that they had chronic hepatitis B. Every patient had liver biopsy procedure to assess the liver pathology. Of the patients who were started entecavir treatment 178 patients had enrolled for this retrospective assesment. All the patients had continuous treatment (0.5 or 1 mg/day) Of these patients 35 were HBeAg positive (21 male, 14 female) and 143 HBeAg negative patients (111 males, 32 female) with chronic HBV infection, treatment initiated starting from 2007 until September 2015. All the follow-ups for liver biochemistry were done every 3 months and HBV DNA assesment was made every 6 months. HBsAg was controlled every 6 months. Total of 9 patients have had HBsAg loss (5.05 %) (3 patients of HBeAg+, and 6 patients HBeAg-) Overall, the mean time to HBsAg loss was 4.1 years \pm 7 months in HBeAg (+) patients and 3.9 years \pm 6 months in HBe Ag (-) group. In this case series, HBsAg loss was observed both in HBeAg positive patients and in HBeAg negative patients. All of the patients with HBsAg loss received entecavir as 0.5 mg. Our results are consistent with the previous reports. Therefore, it may be suggested that treatment with entecavir could be associated to HBsAg loss in a period of time, in both HBeAg positive and HBeAg negative HBV patients.

P-0445

Effect of Telbivudine on intrahepatic cccDNA In HBeAg-positive patients with chronic hepatitis B

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Background/aim: Presence of HBV covalently closed circular DNA (cccDNA) in livers of chronic hepatitis B (CHB) patients is closely associated with persistent viral infection and relapse. The aims of our study were to quantify cccDNA in CHB patients treated with telbivudine (LdT) for 2 years and evaluate the HBeAg seroconversion rate, glomerular filtration rate (GFR) and liver pathology.

Methods: Baseline characteristics for the 24 enrolled patients were as follows: male 88 %, age, mean (years) 37.17 ± 10.77 , body mass index 22.78 ± 3.78 (kg/m²), genotypes B/C 8.3/91.7 %, fatty liver: positive/negative 29.2 %/70.8 %. Patients were treated with monotherapy LdT 600 mg/day for 24-weeks. At this time, patients with HBV-DNA decrease <2 Log, adefovir (ADV) was added; patients with an HBV-DNA decrease >2 Log, LdT monotherapy was continued. Following treatment for 104-weeks, blood biochemistry, viral load, HBV markers, pathology, and imaging were determined.

Results: After 104-weeks of LdT, levels of alanine transaminase (ALT), aspartate transaminase (AST) and alpha-fetoprotein (AFP) decreased; creatine kinase (CK) increased. Compared with baseline, liver inflammation improved significantly ($p < 0.0001$), eGFR increased significantly ($P = 0.022$). Changes in surface antigen were not significant ($P = 0.231$), e antigen ($p < 0.0001$), and HBV-DNA ($p < 0.0001$) were significant. After 104-weeks of treatment, HBV-DNA and cccDNA were both significantly decreased (both $p < 0.0001$). We report no major adverse events.

Conclusions: In our Real World study, LdT-based therapy according to the Roadmap concept successfully achieved important clinical goals. We report significant improvement in liver, renal function markers and virologic and serologic endpoints.

P-0446

Effect of switching to or adding on Ltd on renal function in CHB with suboptimal response to ADV

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Aims: To assess the Glomerular filtration rate (eGFR) in CHB patients treated with AdefovirDipivoxil (ADV) and whether switching to or adding on Telbivudine (LdT) to suboptimal response to ADV has a beneficial effect on renal function.

Methods: This was a prospective cohort study of 80 naïve CHB cases treated with ADV from August 2007 to November 2014 in our hospital. 23 of 80 ADV-naïve patients had completed response, and 57 suboptimal response patients at week 48. 25 of 57 received adding on LdT (Group A) and 32 switched to LdT (Group B). 23 completed response patients continue to ADV monotherapy (Group C). eGFR was assessed every three to 6 months during a mean follow-up of 4 years.

Results: eGFR baseline were not different in three groups ($P = 0.876$). 23.6 % cumulative incidence of eGFR decline for 4 years of ADV monotherapy, including 9 % serious renal dysfunction. During a mean follow-up of 3 years, eGFR was further decline ($P = 0.0027$) in Group C, no significant difference in Group A ($P = 0.089$), and significantly increased in group B ($P = 0.02$) compared with before treatment. eGFR was not significant difference between group A and group B ($t = 1.2750$, $P = 0.2077$), and significant difference compared with group C respectively.

Conclusion: eGFR of ADV monotherapy CHB patients began to decrease at the 1st year, up to 23.6 % patients experienced renal dysfunction after 4 years. LdT could improve eGFR of patients at increased risk for renal impairment with ADV.

P-0447

Efficacy and safety of Telbivudine in treatment naive or ADV treated HBeAg positive CHB patients**Fan Mo, Yao Long, Bing Feng**

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Background/aim: To evaluate the efficacy and safety of telbivudine (LdT) in HBeAg positive CHB patients, who were treatment naive or adefovir (ADV) treated suboptimal responders.**Methods:** We performed a retrospective analysis of 90 HBeAg positive CHB patients. All patients had HBVDNA $>10^3$ copies/ml, ALT $>2 \times$ ULN. 45 patients were naive (group A) and 45 patients were ADV treated suboptimal responders (group B). All patients were treated with telbivudine for at least 104 weeks.**Results:** No significant difference between group A and B regarding baseline ALT and HBV DNA level. 68.89 % (n = 31) and 62.22 % (n = 28) patients in group A and B achieved early response at week 24. At week 104, HBV DNA undetectable rates of two groups were 88.89 and 71.11 %, respectively. HBeAg loss and HBeAg seroconversion rates were 60.00, 37.78 % in group A and 55.56, 22.22 % in group B. Virological breakthrough rates were 6.67, 20.00 %, respectively. At week 104, HBV DNA undetectable, HBeAg loss and seroconversion rates in early responders (n = 59) were higher than those not achieving early response (n = 31), 91.53 vs 58.06 %, 81.36 vs 12.90 %, 40.68 vs 9.68 %, respectively (all $p < 0.05$); resistance rate of early responders was lower (5.08 vs 25.81 %, $p < 0.05$). CK elevation was observed in 60.29 % of patients, but most (78.05 %) of them were transient and mild (CK < 500 U/L).**Conclusions:** The result suggests telbivudine can achieve potent suppression of HBV replication as well as high HBeAg seroconversion in treatment naive patients or ADV treated suboptimal responders. Patients achieve early response at week 24 have a better outcome at week 104.

P-0448

Prepartum use of Telbivudine on preventing mother-to-child transmission of hepatitis B virus**Si Hyun Bae¹, Chung-Hwa Park¹, In-Yang Park², Juyoung Lee³, Sang Bong Ahn⁴, Jung Hwan Shin⁵, Young Min Ahn⁶, Hyun Seung Lee³, Sa-Jin Kim², U Im Chang¹, Chang Wook Kim¹, Se Hyun Jo¹, Young Lee², Jong-Hyun Kim³**¹Department of Internal Medicine, The Catholic University Liver Research Center, The Catholic University of Korea, Seoul, Korea;²Department of gynecology, College of Medicine, The Catholic University of Korea, Seoul, Korea; ³Department of pediatrics, College of Medicine, The Catholic University of Korea, Seoul, Korea;⁴Department of internal medicine, College of Medicine, Eulji University, Seoul, Korea; ⁵Department of gynecology, College of Medicine, Eulji University, Seoul, Korea; ⁶Department of pediatrics, College of Medicine, Eulji University, Seoul, Korea**Aims:** Despite the immunoprophylaxis against HBV, mother-to-child transmission (MTCT) still occurs up to 20 % of babies born from HBV mothers with high viremia.**Methods:** This prospective study was performed to evaluate the efficacy of telbivudine (LdT) on preventing MTCT of HBV and to estimate the sufficient duration of treatment in lowering HBV DNA enough to prevent MTCT. LdT was prescribed to mothers with HBV DNA $>10,000,000$ copies/mL either starting LdT on 28th or 32nd week of pregnancy on mother's choice. Among 27 consented mothers and 2 drop-outs, 12 mothers started LdT on the 32nd week, 13 mothers started on the 28th week and 2 dropped out before starting LdT. Babies underwent immunoprophylaxis as recommended.**Results:** Treatment duration varied from 24 to 94 days with the median of 64 days, and HBV DNA varied from 3,387,240 to 989,000,000 copies/mL with a median of 887,550,000 copies/mL. Regardless of treatment duration or the mothers' HBV status, all mothers successfully experienced HBV DNA dropping $<10,000,000$ copies/mL on the day of delivery, and all the babies showed HBsAb positivity after 7 months of delivery. There were no anti-viral agent related perinatal complications both in mothers and children.**Conclusion:** Taking LdT during third trimester in CHB mothers with high viral load effectively prevented MTCT, and also helped giving rise to the neutralizing antibody in all newborns. These results demonstrate that HBV DNA titer should be included in antenatal check-up list for CHB mothers, and multi-disciplinary approach is needed during perinatal period.

P-0449

The changes of liver stiffness and its associated factors for CHB patients with antiviral therapy**Xu Li, Junqi Niu, Qinglong Jin, Pujun Gao, Hong Zhang**

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Aims: This study aims to assess improvements and linked factors in LSM by transient elastography in Chinese patients with chronic hepatitis B during long-term oral antiviral treatment.**Methods:** From December 2012 to February 2015, we studied 334 consecutive Chinese chronic HBV patients who underwent oral antiviral therapy and received at least two LSM in the first hospital of Jilin University, China.**Results:** In this retrospective study, 334 CHB patients including 201 patients without liver cirrhosis (group 0) and 133 patients with liver cirrhosis (group 1) were enrolled. Each patient received LSM twice, separated by 6 months. Normalized aspartate aminotransferase levels in group 1 was accompanied by a significant reduction of LSM values ($P < 0.001$). Multivariate analysis revealed that higher initial aspartate aminotransferase value, higher follow-up alpha-fetoprotein (AFP) value, lower initial alanine aminotransferase value were associated with a greater decline of LSM value in group 0. Higher initial LSM value and longer antiviral therapy course were correlated with the significant reduction of LSM value, whereas higher follow-up total bilirubin levels contributed to increased LSM value in group 1.

Conclusions: LSM values in liver cirrhosis patients significantly improved and a clear decreasing trend was observed in non-cirrhotic patients after 24 weeks of antiviral drug therapy. In patients without liver cirrhosis, the LSM reduction is related to liver inflammation, whereas it is related treatment durations and serum HBV DNA level in patients with liver cirrhosis. Higher initial LSM value contributed to greater LSM reduction in both group.

P-0450

Assessment of insufficient viral response during entecavir therapy in treatment naïve CHB

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Background: The aim of this study to assess the incidence and outcome of patients with primary non-responder (PNR) or partial virologic responder (PVR) in treatment naïve chronic hepatitis B (CHB) patients receiving entecavir (ETV).

Method: We retrospectively reviewed clinical records of NA-naïve CHB patients who did not have hepatocellular carcinoma before or at ETV 0.5 mg therapy. We enrolled 869 patients who had 6 months ($\leq 2 \log_{10}$ reduction) HBV DNA and received ETV more than 6 months for PNR analysis, and 781 patients who received ETV more than 12 months for PVR (≥ 12 IU/ml at 12 month) analysis.

Results: In 869 patients for PNR analysis, 9 (1.0 %) patients demonstrated PNR. Non adherence rate of patients with PNR showed higher (55.6 vs. 3.6 %, $p < 0.001$) than without PNR patients. In PVR analysis, VR was identified 719 (92.1 %) patients, the cumulative rates of VR at 1, 3, 5 years was 53.7, 86.4 and 91.1 %. Among them, 33 (4.2 %) patients showed poor compliance and achieved lower VR rate than patients with good compliance (66.7 vs. 93.2 %, $p < 0.001$). Two hundred six (26.4 %) patients showed PVR. HBV DNA at 12 month (19 IU/ml) was better predictor for VR than HBV DNA 6 month (AUROC 0.905 vs. 0.865, $P = 0.0076$). Multivariate logistic regression analysis identified high baseline HBV-DNA, HBeAg positivity, and poor compliance showing significant association with PVR.

Conclusion: In CHB patients receiving entecavir, PNR was very rare and PVR was not uncommon but both PNR and PVR were associated with poor compliance.

P-0451

Elevation of ALT during Nuc treatment enhances HBsAg decline

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Background: Serum quantity of HBsAg (qHBsAg) correlates with transcriptionally active cccDNA, which may decline by immune

control. qHBsAg decline during nucleos(t)ide analog (Nuc) treatment is usually small except in patients with ALT $> 5 \times$ ULN. However, 7–9 % of the patients with ALT $< 5 \times$ ULN showed $> 0.5 \log_{10}$ decline. Whether immune mediated ALT elevation during therapy enhances qHBsAg decline requires investigation.

Methods: Sixty-six chronic hepatitis B patients with a baseline ALT $< 5 \times$ ULN and qHBsAg assays at month 6 and 12 of Nuc therapy were included. ALT elevation was defined as > 10 % increase from baseline occurring ≥ 2 weeks after first dose. Serum HBV DNA was measured using Roche Cobas Amplicor HBV Monitor (limit of detection: 20 IU/ml) and qHBsAg using Roche Elecsys[®] II kit (diagnostic range 0.05–52000 IU/mL).

Results: Of these 66 patients, mean age 52.7 ± 11.7 year-old, 78.8 % male, 27.3 % HBeAg positive, the median baseline qHBsAg was 3.2 (1.71–4.71) \log_{10} IU/mL and HBV DNA was 1.24×10^6 (1790– 1.2×10^9) IU/mL. Twelve patients (18.2 %) encountered further elevation of ALT during therapy. These 12 patients had significantly higher baseline HBsAg and HBV DNA levels ($P = 0.048$ and 0.004 respectively). The median reduction at month 6 and 12 in patients with vs without ALT elevation was -0.390 vs $-0.075 \log_{10}$ IU/mL ($P = 0.009$) and -0.530 vs $-0.112 \log_{10}$ IU/mL ($P = 0.000$), respectively. Analyses showed that elevation of ALT during Nuc treatment was an independent factor for greater qHBsAg decline.

Conclusions: In patients with a baseline ALT $< 5 \times$ ULN, further ALT elevation during Nuc therapy, reflecting enhanced immune response, showed a significantly greater HBsAg decline.

P-0452

Regression of HBV-induced Liver Cirrhosis: a preliminary data of a randomized, prospective study

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Aims: To investigate clinical and pathological effects of entecavir alone versus plus thymosin alpha-1 on HBV-induced compensated liver cirrhosis.

Patients and methods: This is an interventional, randomized, controlled, open labeled, multi-center study. Eight hundreds of naive HBV patients ages from 18 to 65 years who histologically confirmed of F4 ($n = 200$) or clinically diagnosed of compensated cirrhosis ($n = 600$), were randomly assigned in a 1:1 ratio. One arm is entecavir alone for 2 years; the other is entecavir for the first 0.5 year, then entecavir plus thymosin alpha-1 for 1 year, entecavir for another

additional 0.5 year. Regression of liver cirrhosis were defined as reduction of 1 point by Ishak score system in liver biopsy or a decrease of 30 % in Fibroscan after 1.5 years therapy.

Results: There were 701 cases have been enrolled for a median treatment duration of 18 months. The HBV-DNA undetectable rates were 81.2, 87.5 and 89.1 % in entecavir group after 6, 12, and 18 months of therapy vs 89, 92.2 and 84.4 % in combination group. Liver stiffness decreased nearly 50 % in both groups. About 40 % of biopsy confirmed and 47 % of clinically diagnosed cirrhosis patients achieved regression of liver cirrhosis. However, there showed no significant differences between entecavir group and combination group after treatment of 18 months. Four patients had varices hemorrhage (1.4 %), five had hepatocellular cancer (1.7 %).

Conclusions: There were significant viral suppression and cirrhosis regression in HBV-induced compensated liver cirrhosis after treatment of entecavir alone or plus thymosin alpha-1.

P-0453

Meta-analysis: geographical variation in treatment rates of chronic hepatitis B (CHB) patients

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Introduction: Globally, CHB affects 240–360 million people, but antiviral therapy can reduce risk of liver cancer and cirrhosis. Our goal is to review overall treatment rates, treatment eligibility and treatment rates in guideline eligible patients.

Methods: Two authors independently searched PubMed, SCOPUS and abstracts from recent liver meetings, bibliographies of selected studies and authors were contacted as applicable. We included studies with >50 CHB patients with reported proportion of patients receiving antiviral therapy. We excluded studies with HCV and HIV co-infection, liver transplant patients and clinical trials. We used random effect model to estimate pooled effect size.

Results: Thirteen studies involving 31,342 patients were included with six US (n = 16,968) and seven non-US studies (n = 14,374). Guidelines applied in US studies included US Panel and AASLD, while US panel, APASL and EASL guidelines were used in others. Overall pooled treatment rate was 33 % (CI 19–48 %). In subgroup analysis of 13,982 untreated patients, 43 % (CI 35–51 %) were found eligible for treatment; and out of these eligible patients, only 41 % (CI 12–71 %) received antiviral therapy, with significantly higher rates (p < 0.001) in US studies (58 %, CI 22–95 %) compared to non-US studies (22 %, CI 14–29 %) (Fig. 1). Treatment rates of AASLD-eligible patients were also higher 70 % (CI 35–105 %) when compared to US panel and other guidelines 32 % (CI 18–45 %) (p < 0.001).

Conclusions: Overall, only 41 % treatment-eligible patients actually received antiviral therapy with even poorer adherence in non-US cohorts. Further efforts are urgently needed to improve the present inadequate linkage to care in CHB patients.

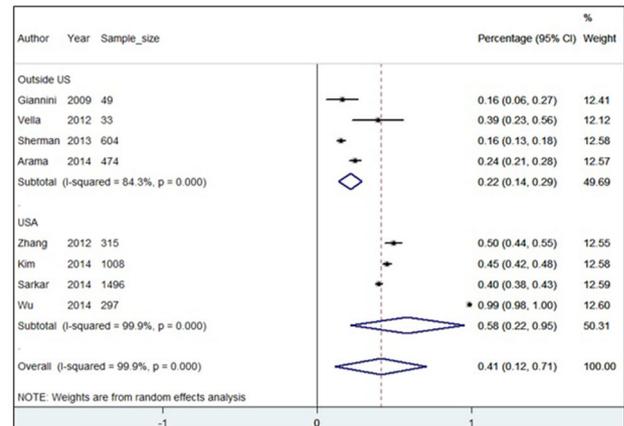


Figure 1: Treatment rates in guideline eligible patients, by study locations.

P-0454

Reversion of liver fibrosis after anti-HBV treatment for 52 weeks is closely related to HBV genotype

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Background and aims: Genetic factors play a major role for treatment response and disease progression of chronic hepatitis B (HBV) infection. However, little was known about the relationship between HBV genotype and the reversion of liver fibrosis after anti-HBV treatment.

Methods: Chronic hepatitis B patients with anti-HBV therapy indications were treated with entecavir for 52 weeks. Liver fibrosis stage was assessed by fibroscan at baseline, 26 and 52 weeks after treatment. The polymerase region of HBV was amplified by nested PCR and directly sequenced. The types of HBV genotype were analyzed using HBVdb database.

Results: Overall, 261 patients from the cohort were available for fibroscan and genotype analysis through week 52. Among these patients, 20.7 % were infected with HBV genotype B, and 79.3 % with genotype C. The baseline fibroscan values were analyzed using t test, and no significant difference was found between genotype B and C (p > 0.05). Entecavir therapy resulted in effective suppression of fibroscan values both at 26 and 52 weeks. In this study, fibroscan value declined by 30 % from baseline was defined as the reversion of liver fibrosis. Interestingly, compared with genotype B (9.3 %), the decline by 30 % in fibroscan value was more pronounced in patients with genotype C (19.8 %, p < 0.05) at 52 weeks after entecavir therapy. However, there was no difference between genotype B (36.9 %) and C (40.2 %, p > 0.05) at 26 weeks.

Conclusions: Through 52 weeks of entecavir therapy, liver fibrosis reverses predominantly in those patients infected with HBV genotype C.

P-0455

The impact of nucleos(t)ides analogues on the renal function in patients with chronic hepatitis B

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Aim: To analysis the dynamic changes of renal function in chronic hepatitis B (CHB) patients with various nucleos(t)ides analogues (NUCs).

Methods: CHB patients in Beijing Friendship hospital between february 2000 and february 2015 were treatment naive or under treatment with NUCs (lamivudine/adefovir/entecavir/telbivudine). Renal function were evaluated by estimated glomerular filtration rate (eGFR, calculated by *CKD-EPI* creatinine equation).

Results: A total of 352 cases were included for a minimum of 2-year follow-up. During treatment, eGFR decreased in entecavir group from baseline in the first (-2.58 ml/min/ 1.73 m², $P = 0.007$), second (-5.30 ml/min/ 1.73 m², $P = 0.002$), third (-6.40 ml/min/ 1.73 m², $P = 0.016$), fourth (-7.68 ml/min/ 1.73 m², $P = 0.005$), fifth (-8.63 ml/min/ 1.73 m², $P = 0.003$) and sixth (-10.39 ml/min/ 1.73 m², $P = 0.001$) year respectively. In adefovir group, eGFR also decreased significantly from baseline in the first (-3.93 ml/min/ 1.73 m², $P = 0.009$), second (-5.38 ml/min/ 1.73 m², $P = 0.009$) and fourth (-9.43 ml/min/ 1.73 m², $P = 0.014$) year respectively. Contrarily eGFR increased in the telbivudine group from baseline in the first (11.70 ml/min/ 1.73 m², $P = 0.000$), second (17.81 ml/min/ 1.73 m², $P = 0.000$) and third (16.11 ml/min/ 1.73 m², $P = 0.009$) year respectively. Change in eGFR were not statistically different in lamivudine group and untreated group.

Conclusions: Long-term treatment with entecavir or adefovir was associated with decreased eGFR in CHB patients, while telbivudine therapy resulted in improved eGFR. Lamivudine therapy had non-significant influence on eGFR.

P-0456

Mutations at rtS202, rtM250 may cause poor viral response to tenofovir rescue in chronic hepatitis B

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Background/aim: Long-term use of nucleos(t)ide analogues (NA) may lead to genotypic and/or phenotypic resistance of hepatitis B virus (HBV). We investigated the efficacy of tenofovir-based rescue therapy in chronic hepatitis B (CHB) patients with newly developed

genotypic resistance to prior NAs or partial virologic response to sequential rescue therapies.

Methods: Fifty-four CHB patients were included retrospectively. The patients were treated with tenofovir alone or combined with lamivudine or entecavir.

Results: There were 26 forms of genotypic resistance at enrollment. The median serum HBV-DNA was 18,438 IU/mL and hepatitis B e antigen (HBeAg) was positive in 83 %. Serum HBV-DNA was undetectable in 50, 61, 76 % of the patients at 3, 6, and 12 months, respectively. In multivariate analysis, HBV-DNA lesser than 20,000 IU/mL and negative HBeAg at baseline were independent predictors of being negative for serum HBV-DNA. Interestingly, rtS202 mutation tended to be associated with an unfavorable response. Other clinical variables and viral resistance genotypes were statistically insignificant in viral response.

Conclusions: Lower serum HBV-DNA, negative HBeAg and lack of rtS202G mutations at baseline may predict favorable response to tenofovir-based rescue therapies in CHB patients with newly developed genotypic resistance to prior NAs or partial virologic response to sequential rescue therapies.

P-0457

Impact of antiviral treatment on clinical outcome after curative therapy for hepatitis B related HCC

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Background: High serum level of hepatitis B virus (HBV) DNA is an independent risk factor of hepatocellular carcinoma (HCC) development and recurrence after curative therapy. We investigated the influence of antiviral treatment after curative therapy of HBV-related HCC on disease free survival and overall survival in patients with HBV replication.

Methods: We retrospectively reviewed the medical records of 203 patients with curative therapy of HBV related HCC from January 2000 to March 2014. The number of patients with HBV DNA level greater than 10⁴ copies/mL without recurrence within 12 months after curative therapy was 39. They were divided into an antiviral treatment group (n = 22) and control group (n = 17).

Results: There was no significant difference in clinicopathologic features between the two groups. Group A had a significantly higher HBV DNA suppression rate (90.9 vs. 11.8 %; HBV DNA PCR < 51 copies/mL) after 12 months of antiviral treatment. HCC late recurrence was observed in 24 of 39 patients during the median observation period of 63 months and this recurrence rate was significantly lower in patients of antiviral treatment group (10 cases, 45.5 % vs. 13 cases, 82.4 %, $P = 0.019$). In multivariate COX regression analysis for disease free survival, DNA suppression was independent prognostic factors ($P = 0.023$). Disease free survival and overall survival were significantly higher in group A (log-rank test; $P = 0.02$, Long-rank test; $P = 0.007$, respectively).

Conclusion: Antiviral treatment after curative therapy of HBV-related HCC reduced the late recurrence and improved disease free survival and overall survival in patients with high serum HBV DNA level.

P-0458

Entecavir treatment for patients with hepatitis B-related cirrhosis is efficacious and safe**Xiangyong Li¹, Xu You², Min Zhang¹, Yuankai Wu¹, Yusheng Jie¹, Guoli Lin¹, Xinhua Li¹, Changhao Zhu¹, Yufeng Zhang¹, Yutian Chong¹**¹The Infectious Disease Department, The Third Affiliated Hospital of Sun Yat-Sen University, Guangzhou, China; ²The Clinical Laboratory Department, The Third Affiliated of Southern Medical University, Guangzhou, Guangdong**Background:** To investigate the efficacy and safety of entecavir (0.5 mg) in ETV naive and lamivudine-pretreated patients with chronic hepatitis B related cirrhosis.**Methods:** We designed a open label and retrospective clinical trial to assess the efficacy and safety of entecavir (0.5 mg) in 103 patients with chronic hepatitis B related cirrhosis. Quantitative HBV DNA measurement 100 IU/ml was used. ALT, HBV DNA and HBeAg were quantitatively measured at baseline and every 3 months.**Results:** 103 patients (78 male) with a median age of 44.1 years (range 25–75 years) were included. 70.0 % (72/103) of patients were ETV naive, and 30.0 % (31/103) had prior exposure to lamivudine (LAM) or adefovir (ADV). At baseline median ALT was 1.41 ULN (range 0.23–8.10 ULN) and HBV DNA was 4.78 log₁₀ IU/ml (range 2.1–7.76 IU/ml). Median treatment duration was 32.6 months (range 3–60 months), without significant clinical side effects. The median HBV DNA level dropped highly significant by 2.19 logs (range 0–5.79 log; $p < 0.001$) and 73.0 % (75/103) patients became HBV DNA undetectable (100 IU/ml). 9.7 % (6/62) of patients achieved HBeAg seroconversion on ETV therapy.**Discussion:** Entecavir therapy can significantly improve liver function and effectively decrease serum level of HBV DNA in patients with chronic hepatitis B related cirrhosis.

P-0459

Long-term outcomes after nucleos(t)ide analogue withdrawal in patients with HBeAg-positive CHB**Li Wang, Yu-Ming Wang**

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Objective: To observe long-term outcomes after NUCs withdrawal in patients with HBeAg-positive CHB.**Methods:** Patients with CHB, who acquired ideal endpoint (HBeAg loss or seroconversion) or satisfactory endpoint (HBeAg seroconversion) after NUCs treatment, in our Department were recruited. All patients were followed-up and monitored by biochemical analysis, HBV markers, HBV DNA level and serological assays.**Results:** A total of 125 cases were recruited, 74.4 % (93/125) cases reached satisfactory endpoint, 25.6 % (32/125) cases achieved ideal endpoint. Consolidation treatment time was significantly different in the two groups ($P = 0.016$), consolidation therapy lasted more than 4 years improved the HBeAg clearance. Besides, it was easier for age older than 30 years and HBV DNA level under 10⁵ IU/ml to obtain HBeAg clearance ($P = 0.003$ and $P = 0.03$, respectively). Duration of following-up was 25 (6–105) months after discontinuation, no relapses occurred in the patients with ideal endpoint. Majority of relapse occurred within 20 (6–58) months after discontinuation. Within 3 year, particularly 1 year, it is the stages of high risk for viral relapse $(P < 0.01)$. SVR in 1, 2, 3 and >3 years was 34.5, 54.2, 54.5 and 93.1 %, respectively.**Conclusion:** After HBeAg seroconversion in patients with chronic hepatitis B, prolong of consolidation therapy can increase ideal endpoint, and more than 3 years of SVR is a predictive marker for longer SVR.

P-0460

Relation of the nucleoside analogues therapy and HBsAg in patient with hepatitis B virus related HCC**Yuri Miyazoe, Naota Taura, Hisamitsu Miyaaki, Kazuhiko Nakao**

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Aims The aim of this study was to clarify the relationship between nucleos(t)ide analog (NA) therapy, serum HBsAg level and HBV DNA level at the time of onset of HCC on patients with hepatitis B.**Methods:** In 69 patients with positive HBsAg who diagnosed our hospital with HCC in 2000–2014. They were categorized into two groups as follows: (1) 16 were classified as the NA group: they received the antiviral therapy with NA, and (2) 53 were put into the non-NA group: they did not receive the antiviral therapy with NA.**Results:** The significance of clinical parameter was examined for the NA group using logistic regression analysis. Univariate analysis revealed ALT >34 IU/l, PLT <121,500/ μ L and HBV DNA level >4 log copies/ml as factors that significantly correlated with NA therapy. Multivariate analysis revealed platelet (OR: 0.12; $P = 0.006$) and HBV DNA level (OR: 0.04; $P = 0.005$) to be independent factors for NA therapy. Furthermore, they were categorized by HBVDNA and HBsAg; low risk group: HBVDNA and HBsAg was lower than 4 log copy/ml and 1000 IU/ml, respectively. The ratio of low risk group in the non-NA group was 34 % whereas in the NA group was 63 %. The NA groups was significantly higher than non-NA group ($P = 0.04312$).**Conclusion:** We found that rate of low HBVDNA and HBsAg in HBV-related HCC patients under the antiviral therapy with NA was higher than in no antiviral therapy patients. In the patients under the antiviral therapy with NA, even if HBVDNA and HBsAg were low, it is important careful monitoring for HCC.

P-0461

Tenofovir added on other nucleoside-analogues was not superior to TDF monotherapy**Mingxing Huang¹, Jingyu Xia¹, Zhongsi Hong¹, Hong Shi², Yutian Chong²**¹Department of Infectious Diseases, the Fifth Affiliated Hospital of Sun Yat-Sen University, Guangzhou, China; ²Department of Infectious Diseases, the Third Affiliated Hospital of Sun Yat-Sen University, Guangzhou, China**Objective:** To compare the clinical efficacy of Tenofovir Disoproxil Fumarate TDF monotherapy and TDF in combined with other nucleoside analogues TDF-combined in the Nucleoside-Analogues NAs experienced chronic hepatitis B patients CHB.**Methods:** 66 NA-experienced CHB patients treated with TDF monotherapy TDF group and 42 with TDF in combined with other NAs TDF-combined group in our follow-up clinic were retrospectively investigated. HBV DNA levels, the rate of

undetectable HBV DNA and the multivariate analysis of HBV DNA negative conversion, ALT normalization rate were evaluated at week 0, 4, 12, 24, 48, 72, 96, 120.

Results: At 24th week, ALT level was significantly lower in TDF group than in TDF-combined group TDF monotherapy: 31.65 ± 12.24 vs. TDF-combined: 44.00 ± 37.60 U/L, $P = 0.045$ $P = 0.045$, while no difference was found in other time. The differences between the two groups in HBV DNA level, the rate of undetectable HBV DNA, ALT normalization rate were not statistically significant all $P > 0.05$. Multivariate analysis of HBV DNA negative conversion showed that difference between TDF and TDF-combined was not statistically significant $P = 0.714$. HBV DNA levels persistent declined and the rate of undetectable HBV DNA continued rise in each group and the differences were statistically significant before in the first 24 weeks all $P < 0.05$, the same as ALT normalization rate in the first 12 weeks treatment all $P < 0.05$.

Conclusions: For CHB patients with NAs experienced, TDF in combined with other NAs was not superior to TDF monotherapy in the HBV DNA inhibition and ALT normalization.

P-0462

Current treatment cost of chronic hepatitis B in China, compared to cost based on standard guideline

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Objectives: Chronic hepatitis B (CHB) is a big health and economic burden in China. To prevent disease progression, CHB treatment is essential; however, patients cannot afford effective treatments. Current cost and practice of CHB treatment are uncertain. The study aims to assess the average cost of CHB care and to compare the current cost to the standardized cost based on the CHB guideline.

Methods: A micro-costing survey was designed and sent to a random sample of tertiary provincial hospitals in the east, central, west, and northwest regions of China. Snowball sampling was utilized to contact tertiary city, secondary city, and secondary county hospitals. A total of 16 hospitals filled out the survey. Average cost per patient per year was assessed and weighted by the proportion of CHB patients. For comparison, standardized cost based on the guideline was also calculated. One-way sensitivity analysis on weights and drug costs was conducted.

Results: The weighted average cost of CHB care was 22,464 CNY. There were variations in drugs and practice among the hospitals. The standardized average cost using TDF was 19,295 CNY per patient per year and 13,234 CNY when using entecavir. Sensitivity analysis revealed that the cost of TDF drastically influenced the total standardized care cost.

Conclusions: CHB care cost is too costly for many. Adhering to guidelines will reduce unnecessary costs; prescribing more effective treatment drugs can prevent CHB from progressing. Further efforts can be made to lower the cost of drugs or increase the reimbursement rate for patients.

P-0463

Quasispecies of antiviral-resistant in naive and undertreated HBV infected patients in Indonesian

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Background: Nucleot(s)ide analog (NA) is the only direct antiviral drug for HBV infection. Its long-term usage leads NA-resistance mutation which mostly present in conserved regions of reverse transcriptase domain (crRTd) of HBV polymerase. The detection of quasispecies by deep sequencing provides opportunities to measure natural occurring mutation and emergence of antiviral resistance. In this study, we examined the profile of HBV quasispecies in crRTd of HBV polymerase including NA-resistance hotspots in naive and undertreated NA-treatment of Indonesian patients with HBV infection.

Methods: 7 patients of each group were involved. To evaluate the amino acid variations and the proportion of HBV quasispecies, the RT domain of PCR product was sequenced using Illumina® Genome Analyzer and analyzed with Genomic Workbench software. The nucleotide alteration resulting amino acid changes was defined as variants.

Results: The HBV subgenotypes of samples were B3, B7 and C1. The rate of amino acid variations in the conserved regions B through E of RT domain was higher in naive NA-treatment (2.2 times). By direct sequencing, NA-resistance mutations were detected only in 1 sample of undertreated NA-treatment. However, ten known NA-resistance variants were detected by deep sequencing in both groups. Overall, rtM204I was the most prominent mutation. There was a slight different proportion at rtV173L, rtT184S, rtS202G and rtN236T ($P > 0.05$).

Conclusion: NA-resistance variants exist prior to treatment and may contribute to the emergence of mutations during treatment. Cohort study is necessary to understand the dynamic of HBV quasispecies on antiviral resistance.

P-0464

An early decrease of HBeAg in CHB patients strongly predicted treatment response during NAs therapy

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Background: To explore early predictors among the HBV markers for the long-term treatment response during nucleos(t)ide analogues (NAs) therapy in patients with HBeAg-positive chronic hepatitis B (CHB).

Methods: Seventy-six treatment-naïve HBeAg-positive CHB patients received NAs optimized therapy (lamivudine and adefovir dipivoxil) for 96 weeks. HBV markers such as HBsAg, HBeAg, anti-HBc and HBV DNA were quantitatively tested every 12 weeks. The logistic (univariable and multivariable) analyses were performed to identify response predictors.

Results: Forty-three CHB patients (56.6 %) achieved virological response (HBV DNA <300 copies/ml) and 15 patients (19.7 %) developed HBeAg seroconversion after the 96-week treatment. The HBeAg reduction from the baseline to week 12 independently predicted HBeAg seroconversion (OR = 2.37, P = 0.006) and virological response (OR = 2.87, P = 0.006) in patients after the 96-week therapy, and the area under the receiver operating characteristic curve (AUROC) was 0.68 and 0.69, respectively. In addition, the HBeAg reduction from the baseline to week 24 also independently predicted HBeAg seroconversion (OR = 2.53, P = 0.018) and virological response (OR = 3.24, P = 0.012) in patients after the 96-week therapy, and the AUROC was 0.83 and 0.78, respectively. The HBeAg titer decreased by \log_{10} S/CO at week 24 predicted HBeAg seroconversion and virological response with a sensitivity, specificity, positive predictive value, negative predictive value of 80.0, 75.0, 44.0, 93.9 and 59.5, 93.9, 92.7, 64.0 %, respectively.

Conclusions: The declined level of HBeAg at week 24 strongly predicted virological response and HBeAg seroconversion in HBeAg-positive CHB patients after the 96-week NAs treatment and may be used as a reference parameter to optimize the NAs therapy.

P-0465

Baseline vitamin D level predict virologic response in hepatitis B patients treated with telbivudine

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Background: To explore the association between baseline vitamin D level and treatment outcome in Chronic hepatitis B (CHB) patients.

Methods: Five hundred sixty patients from a multicenter, randomized, controlled study who completed 104-week treatment with telbivudine or telbivudine plus adefovir were included in this study. Baseline serum 25(OH)D levels were determined using an automated electrochemiluminescence-based assay, Elecsys Vitamin D Total (Roche Diagnostics, Mannheim, Germany). Univariate and multivariate analyses were conducted to determine the association between baseline parameters and week-104 treatment outcome.

Results: The mean 25-hydroxyvitamin D value was 29.64 ng/ml. The percentage of patients with vitamin D insufficiency (<30 ng/ml) and vitamin D deficiency (<20 ng/ml) were 55 and 20.9 %, respectively. The mean 25(OH)D level increased gradually from North China, Central China, to South China (35.95 ± 11.10 vs. 29.06 ± 10.58 vs. 24.44 ± 9.62 ng/mL, $p < 0.001$). Patients with sufficient vitamin D (> 30 ng/ml) achieved higher virologic response rate than those with vitamin D insufficiency (81.7 vs. 67.2 %, $p < 0.001$). The area under the curve of vitamin D to predict virologic response is 0.65 ($p < 0.001$, 95 % CI 0.62–0.67). On multivariate analysis, vitamin D levels

was independent predictor of virologic response, but not associated with HBeAg seroconversion or ALT normalization.

Conclusions: Vitamin D insufficiency was highly prevalent in treatment naïve CHB patients in mainland China. Baseline vitamin D levels can predict virologic response, but not HBeAg seroconversion or ALT normalization.

P-0466

Hepatitis B surface antigen monitorization during oral antiviral therapy

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Background: The purpose of our study was to investigate if there is any use of monitoring serum HBs Ag levels during oral antiviral drug therapy.

Method: A total of 50 treatment naïve patients with chronic hepatitis B infection (19/31 F/M) were enrolled to our study. HBeAg was positive in 11 patients, while it was negative in 39 patients. Ten patients received tenofovir, 10 entecavir, 12 lamivudine and 18 telbivudine. Serum HBV DNA and HBsAg were recorded at 0, 3rd, 6th and 12th months of treatment. Serum HBsAg level was measured by using ELISA and HBV DNA by PCR methods.

Results: Fibrosis scoring was made in each patient according to Ishak's scoring and there was no correlation with fibrosis stage and HBs Ag or HBV DNA levels. Serum HBV DNA and HBsAg levels were found to be significantly higher in HBeAg positive than HBe Ag negative patients. Although there was a positive correlation between basal serum HBV DNA and HBsAg levels, this disappeared during 3rd and 6th and 12 months of therapy in all treatment groups. A significant reduction was not observed at 3rd month in serum HBsAg levels in all patients, while at the end of 6 and 12th months, serum HBs Ag decline was significantly higher in tenofovir (80 %) and entecavir (79 %) groups than in the telbivudine (46 %) and lamivudine (38 %) groups.

Conclusion: On contrary to peginterferon treatment, HBsAg monitorization does not provide additional benefit to HBV DNA measurement during oral antiviral therapy.

P-0467

The changes in the balance of Treg and Th17 after entecavir therapy

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Objective: To explore the changes in the balance of Treg and Th17 after entecavir therapy by researching IL-17, IL-10 and TGF- β 1.

Method: Gathering the clinical data of 20 healthy adults and 35 patients with HBeAg-positive chronic hepatitis B who had used

entecavir for the initial treatment during January 2012 to December 2012. The patients were divided into two groups as follows: ETV group and control group. All the patients in two groups were analyzed for their clinical items including Interleukin-17, Interleukin-10, transforming growth factor-beta 1.

Result: After 48 weeks' treatment with entecavir, the ratio of IL-10/IL-17 declined when we compared with the ratio before treatment, but there was little change in the ratio of TGF- β 1/IL-17 before and after treatment. The two ratios are all lower than the ratio of the healthy control group.

Conclusion: After 48 weeks' treatment with entecavir, the balance between Treg and Th17 tend to the side of Th17.

P-0468

Tenofovir and entecavir for patients with chronic hepatitis resistant to adefovir and entecavir

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Background/aims The aim of this study was to evaluate the efficacy of ETV and TDF combination therapy and associating factor of virologic response in CHB patients who showed suboptimal response to ETV and adefovir (ADF).

Methods: A total of 34 CHB patients who did not achieve completed virologic response (CVR, serum HBV DNA <116 copies/mL) after treating with ETV and ADV over 12 months were treated with ETV and TDF. Baseline genotypic resistance profiles were analyzed before rescue therapy. Biochemical and virologic parameters were analyzed at baseline 3, 6, 9, and 12 months.

Results: Cumulative number of patients with CVR after 3, 6, 9, and 12 months was 11 (32.4 %), 20 (58.8 %), 22 (64.7 %) and 23 (67.6 %), respectively. In baseline factor associated with a CVR at 12 months, baseline HBV DNA level (3.54 ± 0.80 vs 4.50 ± 1.44 copies/mL, $P = 0.017$), virologic response after 6 months (2 log decrease from the baseline, 35.4, 4.3 %, $P = 0.014$), and proportion of ADV resistant mutation (27.8 % vs 72.7 %, $P = 0.018$) is high in patient without CVR. Baseline HBV DNA levels, ADV resistant mutation were predictive of the CVR rate after 12 months in multivariate analysis (OR = 10.62; CI 1.20–93.808; $P = 0.033$, OR = 64.02; CI 1.39–4175.16; $P = 0.034$, respectively).

Conclusion: In CHB patients with suboptimal response to ETV and ADF, treatment with a combination of ETV and TDF induced relatively good virologic response. CVR rate after 12 months of treatment is associated with ADV resistant mutation and DNA level at baseline.

P-0469

Treatment efficacy of tenofovir in treating drug-resistant chronic hepatitis B

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Background/aims: The purpose of this study is to evaluate the predictive factors by examining the treatment effects and responses in patients with chronic hepatitis B after a 6-month period of tenofovir rescue therapy.

Methods: The medical records of 68 patients who had undergone a 6-month treatment of tenofovir were analyzed. Those patients had previously been identified with drug resistance mutations from September 2012 through August 2014. The virological response showed a decrease in a value of less than 20 IU/mL of HBV DNA and the Partial virological response showed a decrease in value over a 1log baseline of HBV DNA, while throughout the trial, HBV DNA was continuously detected.

Results: After 24 weeks 36 patients (53 %) showed a virologic response, and 29 patients (43 %) showed a partial virological response. Compared with virological response patients, the partial virological response patients showed a higher HBeAg positive rate with the baseline and showed a significant difference ($p < 0.001$). The results of the analysis showed that the low amount of HBV DNA ($p < 0.001$) and serum HBeAg-negative status ($P = 0.004$) was independent factor to predict virological response at 24 weeks after tenofovir rescue therapy.

Conclusions: Tenofovir rescue therapy showed a positive therapeutic effect in drug-resistant chronic hepatitis B patients. And baseline low HBV DNA levels and HBeAg negative status is useful in predicting the virological response of tenofovir rescue therapy.

P-0470

Optimized therapy with domestic lamivudine and adefovir in HBeAg-positive chronic hepatitis B

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Background and aims: The drug resistance is quite common in patients with chronic hepatitis B (CHB) during long-term lamivudine (LAM) monotherapy. The optimized therapy with domestic LAM and adefovir dipivoxil (ADV) was used to treat HBeAg-positive CHB patients and efficacy was evaluated.

Methods: Ninety three CHB patients were treated with LAM and added on ADV when serum HBV DNA level >300 copies/ml at week 24 or the virological breakthrough was observed during LAM monotherapy. The antiviral efficacy was evaluated every 12 weeks during 96-week follow-up. The intrahepatic HBV tDNA and HBV cccDNA levels were quantitated by real-time PCR at the baseline and week 96. The drug resistant mutants were detected at the baseline and virological breakthrough.

Results: Seventy eight patients completed the 96-week follow-up study, 56.4 % of patients achieved virological response (HBV-DNA <300 copies/ml). HBeAg loss and HBeAg seroconversion rates were 24.4 and 19.2 %. HBsAg loss and HBsAb seroconversion rate was 1.3 %. ALT and AST normalization rates were 87.2 and 93.6 %. Intrahepatic HBV tDNA declined from 2.63 ± 0.94 to 0.55 ± 0.73 log₁₀ copies/cell and HBV cccDNA dropped from 0.56 ± 0.90 to -0.98 ± 0.56 log₁₀ copies/cell. Paired analysis of liver biopsies, 48.3 % of patients achieved fibrosis improvement and 71.7 % were

reduced in necro-inflammation. LAM and ADV-related resistance rate was 8.6 %.

Conclusion: Through 96-week optimized antiviral therapy in CHB patients, HBV replication is effectively inhibited in the liver, liver function, inflammation and fibrosis are improved, and drug resistance mutation rate was lower. Key words: CHB; HBV DNA; HBV cccDNA; antiviral therapy

P-0471

De novo LAM and ADV combination treatment in CHB patients with high HBV DNA level is efficacious

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Background: Aim of this study is to investigate the efficacy and safety of de novo lamivudine combined adefovir dipivoxil in chronic hepatitis B patients with high HBV DNA level.

Method: We designed an open label and retrospective clinical trial to assess the efficacy and safety of de novo lamivudine combined adefovir dipivoxil in 33 patients with chronic hepatitis B. Quantitative HBV DNA measurement below 100 IU/ml was used. ALT, HBV DNA and HBeAg were quantitatively measured at baseline and every 3 months.

Results: 33 patients (28 male) with a median age of 34.7 years (range 23–67 years) were included. At baseline median ALT was 3.34 ULN (range 2.32–11.7 ULN) and HBV DNA was 6.18 log₁₀ IU/ml (range 6.0–8.2 IU/ml). Median treatment duration was 38.3 months (range 36–50 months), without significant clinical side effects. The median HBV DNA level dropped highly significant by 5.19 logs (range 4.1–6.1 log; p below 0.001) and 87.9 % (29/33) patients became HBV DNA undetectable (below 100 IU/ml). 9.4 % (3/32) of patients achieved HBeAg seroconversion on de novo lamivudine and adefovir dipivoxil combination therapy.

Discussion: De novo lamivudine and adefovir dipivoxil combination therapy can significantly improve liver function and effectively decrease serum level of HBV DNA in chronic hepatitis B patients with high HBV DNA level.

P-0472

HBsAg decline after pegylated-interferon-alpha in HBeAg positive with nucleoside maintenance

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Background and aims: The aim of this study is to investigate pegylated interferon (PI) after long term NA therapy might potentiate HBeAg seroconversion and eventually HBsAg loss and/or seroconversion.

Methods: The patient with HBeAg-positive CHB who had been treated with any NA except telbivudine, who have an undetectable HBV DNA (<400 copies/mL) at least 1 year, were randomised 1:1 to receive PI alfa-2a 180 ug/week or previous NA for 48 weeks. The primary endpoint was change in log₁₀ HBsAg titer during antiviral therapy (ClinicalTrials.gov: NCT01769833). Interim analysis was performed after 6 months of treatment.

Results: Until interim analysis, 89 patients were randomized; 49 received PI study drug dose. Four patients were excluded due to screen failure. At 24 week, on treatment HBsAg decline was significantly higher in patients who switched to PI alfa-2a than those with NA (mean log HBsAg plusmn SD 0.248 ± 0.404 vs. -0.065 ± 0.321; p < 0.001). Only patients receiving PI alfa-2a achieved HBeAg seroconversion [23.5 % (8/34) vs 0 % (0/24); p = 0.016]. Until week 24, HBsAg loss was not observed in both groups. On-treatment HBV DNA elevation rate (more than 1000 copies/mL) was significantly higher in patients who switched to PI alfa-2a than those with NA [37.5 % (12/32) vs. 8 % (2/25); p = 0.013]. However, none of them was accompanied by elevated level of alanine aminotransferase. PI alfa-2a was well-tolerated.

Conclusions: This interim analysis showed that, for patients who achieve virological suppression with oral NA, switching to a 24 weeks PI alfa-2a treatment significantly decrease HBsAg titer and increases HBeAg seroconversion.

P-0473

Eight years of PEG INF and Entecavir therapy on HBsAg loss and seroconversion with chronic HBV

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Introduction: HBV is the most common viral infection disease in the world influencing nearly 2 billion people. Approximately 400 million people worldwide are infected with HBV.

Objective: Our objective is to evaluate the long-term efficacy of combination therapy with pegylated interferon alfa-2a (PEG-IFN alpha-2a) and entecavir on HBsAg loss and anti-HBs development in patients with chronic active hepatitis.

Methods: Overall, 17 patients were determined to be HBeAg negative while 10 were HBeAg positive. PEG-INF alpha-2a 180 mcg/day and Entecavir 0.5 mg/day were administered all of the patients on a weekly basis. Afterwards, patients were asked to attend the outpatient control visit performed in every 6 months until the end of 7 year follow up period. Time HBV-DNA negative and HBsAg loss and Anti-HBs positivity seroconversion recorded.

Results: Patients showing HBsAg loss was determined to increase 8 (29.6 %) in the 3rd years of the treatment while seroconversion to anti-HBs was evident in 7 (25.9 %) patients in the 4th year of the combination treatment. Seven year of the combination therapy patients showing HBsAg loss was determined to increase 9 (33.3 %) while seroconversion to anti-HBs positivity was evident 8 (29.6 %) patients. End of 4 years of therapy HBV-DNA negativity reached 100 %.

Conclusions: Our study findings gradual increase in the ratio of both HBsAg loss and anti-HBs seroconversion within the 7 years follow-up, the combination antiviral therapy of PEG-INF alpha 2a plus entecavir seems to provide a long lasting efficacy in cases chronic HBV.

P-0474

HBsAg and cccDNA reduction in patients with CHB receiving PEG-IFN with or without entecavir**Natthaya Chuaypen, Sunchai Payungporn, Pisit Tangkijvanich**

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Background: Current data have demonstrated that pegylated interferon (PEG-IFN) treatment in patients with chronic hepatitis B (CHB) results in the reduction of serum HBsAg and intrahepatic covalently closed circular DNA (cccDNA) levels. However, it is unclear whether a combination with entecavir (ETV) might lead to greater decline of these viral markers compare with PEG-IFN alone.

Method: Seventy-two patients with HBeAg-negative CHB were enrolled. Among these, 35 and 37 patients were treated with PEG-IFN monotherapy (group I) and PEG-IFN plus ETV (group II) for 48 weeks and followed-up for additional 48 weeks. Paired liver biopsies at baseline and the end of treatment were analyzed for cccDNA by real-time PCR. Serum quantitative HBsAg was measured by a commercially available assay.

Results: Virological response (HBV DNA <2000 IU/mL at week 96) was achieved in 34.3 and 37.8 % of groups I and II, respectively. At baseline, groups I and II had comparable mean levels of serum HBsAg (3.4 ± 0.4 vs. 3.5 ± 0.4 log₁₀ IU/mL, $P = 0.405$) and cccDNA (0.4 ± 1.0 vs. 0.5 ± 1.3 log₁₀ copies/cEq, $P = 0.698$). At the end of treatment, the corresponding groups also had comparable mean decline of serum HBsAg (1.1 ± 1.1 vs. 0.8 ± 0.9 log₁₀ IU/mL, $P = 0.294$) and cccDNA (0.9 ± 1.3 vs. 1.2 ± 1.8 log₁₀ copies/cEq, $P = 0.390$). Baseline serum HBsAg was not correlated with cccDNA ($r = 0.046$, $P = 0.700$), but HBsAg reduction at the end of treatment was positively correlated with decreased cccDNA levels ($r = 0.332$, $P = 0.004$).

Conclusion: Both regimens had similar treatment response. The combination therapy did not lead to greater decline of HBsAg and cccDNA levels over PEG-IFN monotherapy.

P-0475

Nucleotide analogues improve interferon responsiveness in HBV-infected human hepatocytes**Masataka Tsuge^{1,2,3}, Nobuhiko Hiraga^{1,2}, Michio Imamura^{1,2}, Hiromi Abe^{1,2}, Daiki Miki^{1,2}, Hidenori Ochi^{1,2}, C. Nelson Hayes^{1,2}, Kazuaki Chayama^{1,2}**

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Background: Interferon treatment can reduce HBsAg in a subset of chronic hepatitis B patients. One cause of this limitation is thought to be that interferon responsiveness in human hepatocytes is suppressed by HBV infection. In the present study, we analyzed whether the suppression of HBV replication by nucleotide analogue (NA) treatment could improve interferon responsiveness in HBV-infected human hepatocytes.

Methods: Thirty-seven chronic hepatitis B patients were enrolled. Twenty of 37 patients underwent sequential interferon therapy prior

to NA therapy, and the remaining 17 patients underwent interferon mono-therapy. Serum HBsAg titers were measured every year until 5 years after interferon therapy. To confirm the clinical data, in vitro experiments were performed, and interferon responsiveness was evaluated by induction of interferon stimulated genes (ISGs).

Results: In the clinical study, more than 1 log IU/ml reduction of HBsAg titer was achieved in 11 of the 37 patients, and the following factors were found to be associated with HBsAg reduction: gender, HBsAg level, the existence of HBeAg, and prior NA therapy ($P = 0.007$, $P = 0.027$, $P = 0.031$, $P = 0.037$, respectively). Therefore, interferon responsiveness could be improved by prior NA therapy. In vitro, MxA and OAS1 were significantly induced by interferon treatment in the absence of HBV (19.2- vs. 9.7-fold, respectively). However, ISG induction by interferon treatment was suppressed in HBV-infected cells (10.3- vs 4.83-fold). After NA treatment, interferon-induced ISG expression was restored (13.5-, 5.73-fold, respectively). Furthermore, ISG induction may be suppressed by the large-HBs protein.

Conclusions: Prior NA therapy could improve interferon responsiveness in HBV infected human hepatocytes.

P-0476

Sequential combination of PegIFN in ETV-experienced patients and PegIFN in treatment-naive patients**Yiqi Yu¹, Guojun Li², Shaolong Chen¹, Jing Wang¹, Lingyun Shao¹, Wenhong Zhang^{1,3,4}**

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This study analyzed the efficacy of sequential combination therapy of PegIFN in ETV-experienced patients and compared to treatment-naive patients receiving PegIFN. This was a matched retrospective cohort study. Treatment-naive HBeAg-positive patients receiving PegIFN as well as ETV-experienced patients adding on PegIFN (S-C therapy, $n = 81$) for 48 weeks were retrospectively enrolled. A matched-pair was created at a 1:1 ratio from each treatment group according to gender and age (<5 years). Rate of HBeAg seroconversion and HBsAg loss between the two groups were compared at the end of 48-week treatment. A total of 130 HBeAg-positive patients were included, with 49 treatment-naive patients treated with PegIFN monotherapy and 81 ETV-experienced patients treated with S-C therapy. The matched-pair consisted of 48 patients in each treatment group. After 48 weeks of treatment, 19 (39.6 %) patients treated with S-C therapy achieved HBeAg seroconversion compared to 13 (27.1 %) patients treated with PegIFN. The difference between the two groups did not reach statistical significance ($P = 0.2790$). Rate of HBsAg loss was similar between PegIFN and S-C groups (10.4 vs. 6.3 %, $P = 0.7145$). Multivariate analysis indicated that baseline HBeAg level and HBeAg decline at week 12 were independent factors associated with HBeAg seroconversion. Baseline HBsAg level and HBsAg decline at week 12 were independent factors associated with HBsAg loss. **Conclusions:** Patients who did not achieve serological responses during previous ETV therapy can achieve serological responses after receiving sequential combination therapy. The response rates were comparable to treatment-naive patients. Patients with lower baseline HBeAg/HBsAg levels or more pronounced decline at week 12 were more likely to achieve responses.

P-0477

Comparison of Asians vs. non-Asians treated with TDF plus peginterferon (PEG)

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Background: Differences in viral and patient characteristics between Asians and non-Asians may impact response to HBV therapy. We examined differences in between Asian and non-Asian patients enrolled in the Tenofovir Disoproxil Fumarate (TDF) plus PEG combination study GS-US-174-0149.

Methods: 740 CHB patients without advanced liver disease were randomized 1:1:1:1 to receive TDF+PEG × 48 weeks (Arm A); TDF+PEG × 16 weeks followed by TDF × 32 weeks (Arm B); continuous TDF (Arm C); PEG × 48 weeks (Arm D). Baseline characteristics, Week 72 efficacy and overall safety were compared between Asians and non-Asians. Primary efficacy was HBsAg loss at week 72.

Results: Of 554 Asian patients randomized, most were male and HBeAg-positive, and predominantly genotype (Gt)-B/C; while among 186 non-Asian patients, most were male, HBeAg-negative and predominantly Gt- A/D (Table 1). Overall, rates of HBsAg decline $\geq 1 \log_{10}$ IU/mL from baseline to week 48 were lower among non-Asians (11 %) vs. Asians (26 %) ($p < 0.001$) (Table 1). Rates of HBsAg loss at Week 72 were 7.4, 2.3, 0, and 3.1 % in Asians compared to 14.6, 4.4, 0, and 2.2 % in non-Asians for Arms A-D, respectively ($p > 0.05$ for Asian vs non-Asian comparison within each treatment group). Within each treatment arm, no significant differences were observed for rates of normal ALT, HBeAg loss/seroconversion between Asian and non-Asian patients at Week 48. Rates of treatment-emergent AEs, discontinuations and SAEs were similar between Asians and non-Asians.

Conclusion: Although more Asians had $>1 \log_{10}$ HBsAg decline from baseline, no statistically significant differences in efficacy were observed between Asians and non-Asians.



Table 1.	Asians (N=554)	Non-Asians (N=186)	P-value
Male (%)	63.7	71.5	0.061
HBeAg-positive (%)	64.8	37.1	<0.001
Viral Genotypes (%)			<0.001
Gt A	2.3	25.8	
Gt B	35.7	2.7	
Gt C	56.3	1.1	
Gt D	5.4	66.7	
Other	0.18	3.8	
HBsAg decline $>1 \log_{10}$ from baseline to Week 48 (%)	26.0*	11.1*	<0.001
HBsAg loss at Week 72 (Arms A:B:C:D) (%)	7.4: 2.3: 0: 3.1	14.6: 4.4: 0: 2.2	N.S.

*Among 497 Asian and 162 Non-Asians who reached Week 48.

N.S.=Not Significant

P-0478

The pharmacoeconomics value for HBeAg-positive CHB patients to switch from NUCs to PEG-IFN- α 2a

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Background: This study aimed to evaluate pharmacoeconomic-value of regimens in CHB-patients who switched from a long-term treatment of NUCs to Pegylated interferon- α -2a (PEG-IFN- α -2a). **Methods:** Pharmacoeconomic studies would provide evidence for clinical-practices, because treatment cost is always one of the critical concerning factors for drug-therapy choices. A Markov-model was applied to simulate the long-term treatment-costs and health-status changes when patients receiving PEG-IFN- α -2a and NUCs regimens. The clinical-data were collected from a randomized-controlled-trial NEW-SWITH study. The real-world cost-data, including drugs/treatment-fees, were collected through expert-interviews.

Results: In NEW-SWITH study, 303 HBeAg-positive-CHB patients (HBV DNA <200 IU/ml and HBeAg-loss) with a prior NUCs-history were included, and the HBsAg-loss-rate 16.2 % and HBeAg-seroconversion-rate 56.4 % were observed. For 124 sub-PEG-IFN-group-patients (HBV DNA <200 IU/ml, HBeAg-loss and HBsAg <1500 IU/ml), significantly-higher HBsAg-loss-rate 33.1 % and HBeAg-seroconversion-rate 58.7 % were observed. The Markov-model demonstrated that, compared with NUCs-regimen, PEG-IFN- α -2a-regimen could bring incremental 3.13 QALYs (quality-adjusted-life-years) at additional-costs USD3,253. For subgroup-patients, it could bring higher-incremental 3.64 QALYs at lower-additional-costs USD2,384. It cost PEG-IFN- α -2a-regimen USD38,982 and USD21,982 to fully-achieve HBsAg-loss and HBeAg-seroconversion. For NUCs-regimen, these unit-cost would be USD586,607 and USD55,340. The PEG-IFN- α -2a-regimen is cost-saving to achieve primary-point-per-case.

Conclusions: The cost-per-QALY-gained of PEG-IFN- α -2a-regimen are USD1040 and USD654 for overall and subgroup respectively, lower than 2014 China GDP per-capita (USD7270). Based on WHO's cost-effectiveness-threshold, for HBeAg-positive CHB-patients who had received 2-year NUCs-treatment, switch to 48-week PEG-IFN- α -2a treatment-regimen represents a justifiable-strategy on both clinical and economic perspective.

P-0479

48 weeks follow-up in CHB patients after NAs treatments cessation

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Background: The criteria for nucleos(t)ide analogues cessation in CHB patients is still unclear. We aimed to find out the feasibility and safety of NAs treatments cessation.

Methods: NAs treatments were stopped in patients treated for ≥ 2 - years with undetectable HBV DNA levels on ≥ 3 separate occasions 6 months apart before treatments cessation. Otherwise, HBeAg seroconversion ≥ 1 year was required in HBeAg positive patients. Clinical data were recorded at the time of and after NAs cessation.

Virologic relapse was defined as HBV DNA >2000 IU/mL, while clinical relapse was defined as HBV DNA >2000 IU/mL and ALT >2 \times ULN. NAs retreatment would be performed when clinical relapse occurred.

Results: 58 patients were recruited and 36 patients finished 48 weeks follow-up. None of them developed into cirrhosis, liver failure or HCC. 5 patients got negative HBsAg when NAs cessation and their HBV DNA remained undetectable. The cumulative rates of virologic relapse, clinical relapse and retreatment were shown in Fig. 1. 14 patients were retreated with NAs and they got normalized ALT and undetectable HBV DNA within 24 weeks. There were no statistical differences in age, duration of NAs treatment, duration of negative HBV DNA maintenance and level of HBsAg when cessation between non-relapse group and virologic relapse group in both HBeAg positive and negative patients (Tables 1, 2).

Conclusions: NAs cessation in CHB patients is safe with strict follow-up. Virologic relapse and clinical relapse increase in the course. Negative HBsAg is a predictive factor for NAs cessation.

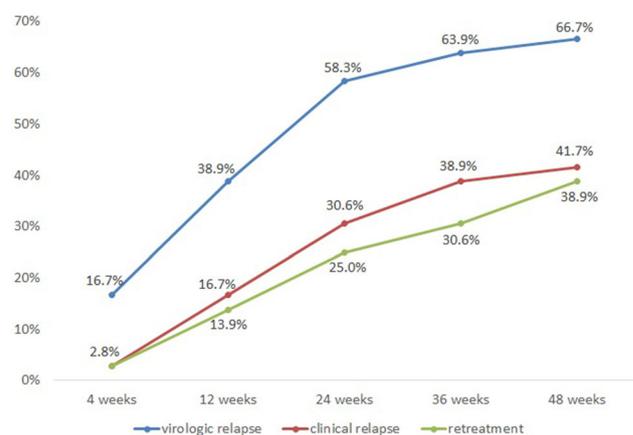


Figure 1 the cumulative rates (n=36)

Table 1 Predictive factors for HBeAg positive patients

	Non-relapse n=9	Virologic relapse n=11	F	p
Age(year)	30.4 ± 13.1	36.6 ± 6.5	1.844	0.191
Duration of NAs treatment(year)	4.89 ± 2.79	3.40 ± 1.11	2.648	0.121
Duration of negative HBV DNA maintenance(year)	4.13 ± 2.38	2.86 ± 1.09	2.501	0.131
Duration of HBeAg seroconversion maintenance(year)	2.90 ± 2.06	2.13 ± 1.03	1.197	0.288
Level of HBsAg(IU/ml)	1402.20 ± 2133.26	2476.78 ± 2005.09	1.343	0.262

Table 2 Predictive factors for HBeAg negative patients

	Non-relapse n=3	Virologic relapse n=13	F	p
Age(year)	33.3 ± 7.5	44.9 ± 8.5	4.614	0.050
Duration of NAs treatment(year)	3.60 ± 1.04	4.24 ± 1.68	0.387	0.544
Duration of negative HBV DNA maintenance(year)	3.60 ± 1.42	3.48 ± 1.30	0.021	0.886
Level of HBsAg(IU/ml)	1677.23 ± 2831.12	2610.88 ± 6039.39	0.066	0.802

*P-0480***Long term follow up of chronic hepatitis B patients after oral antiviral treatment discontinuation****Seong Hee Kang, Jong Eun Yeon, Young Sun Lee, Tae Suk Kim, Yang Jae Yoo, Ji Hoon Kim, Kwan Soo Byun**

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Introduction: In the current guidelines, 6–12 months of consolidation after the HBeAg seroconversion is recommended for HBeAg-positive patients. And the ideal treatment duration of HBeAg negative chronic hepatitis B (CHB) is not well known. We evaluated long term clinical courses in patient after antiviral treatment cessation.**Methods:** A total of 118 HBeAg-positive and 60 HBeAg-negative CHB patients who discontinued lamivudine between 1997 and 2014 were analyzed.**Results:** In HBeAg-positive patients, the mean duration of lamivudine treatment in was 32.3 months and the mean follow-up period after discontinuation was 72.4 months. The cumulative probability of a composite relapse ($\geq 10^4$ copies/mL) at 1, 6, 12, 24, 48, 96, 180 months were 11.9, 28.8, 38.1, 51.7, 59.3, 63.6, 64.4 % respectively. Multivariate analysis revealed pretreatment age ≤ 34 years [HR 0.324, $P = 0.005$] and undetectable HBV DNA [HR 0.261, $P = 0.003$] within the three month after treatment discontinuation were the predictive factors of non-relapse. In HBeAg-negative patients, the mean age of the patients was 39.2 years. The cumulative probability of a composite relapse were 25.0, 33.3, 35.0, 41.7, 43.3, 46.7, 48.3 % respectively. Mean time to relapse after off-treatment was 13.6 months. The mean duration of lamivudine treatment were 29.8 and 33.5 months in the relapse and non-relapse group ($P = 0.447$).**Conclusions:** Even with the long term consolidation after HBeAg seroconversion, most HBeAg positive CHB patients showed relapse. And half of patients with HBeAg-negative CHB had relapsed after treatment cessation. Clinicians should maintain antiviral therapy for long term period or until HBsAg was lose.*P-0481***24 weeks follow-up in CHB patients after NAs treatments discontinuation****Wenxiang Xu, Liang Peng, Zhanlian Huang, Chuming Chen**

The Third Affiliated Hospital of Sun Yat-sen University, Guangzhou, Guangdong, China

Background: The criteria for nucleos(t)ide analogues discontinuation in CHB patients is unknown. We aimed to find out the relapse rate and predictive factors of NAs treatments discontinuation.**Methods:** NAs treatments were stopped in patients treated for ≥ 2 years with undetectable HBV DNA levels on ≥ 3 separate occasions 6 months apart before treatments cessation. Otherwise, HBeAg seroconversion ≥ 1 year was required in HBeAg positive patients. Clinical data were recorded at the time of and after NAs cessation. Virologic relapse was defined as HBV DNA >2000 IU/mL, while clinical relapse was defined as HBV DNA >2000 IU/mL and ALT $>2 \times$ ULN.**Results:** 60 patients were recruited, 48 patients finished 24 weeks follow-up. 9 patients got negative HBsAg when NAs cessation and their HBV DNA remained undetectable. The cumulative rate of

virologic relapse at 4, 12 and 24 weeks was 16.7, 39.6 and 56.3 %, respectively. The cumulative rate of clinical relapse at 4, 12 and 24 weeks was 2.1, 14.6 and 25 %, respectively. There were no statistical differences in age, duration of NAs treatment, duration of negative HBV DNA maintenance and level of HBsAg when cessation between non-relapse group and virologic relapse group in both HBeAg positive and negative patients.

Conclusions: Virologic relapse and clinical relapse increase in the course of NAs discontinuation. Negative HBsAg is a predictive factor for NAs discontinuation. No other predictive factors can be found in this study.*P-0482***Durability of telbivudine efficacy off-treatment in chronic hepatitis B patients****Liang wu, hanxin shen**

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Background/Aims: The aim of our study was to evaluate the sustained efficacy and relapse rate following withdrawal of telbivudine (LdT) in chronic hepatitis B patients.**Methods:** We retrospectively analyzed (2007–2015), 59 HBeAg-positive patients treated with LdT whose therapy was withdrawn. Included in our analysis are patients who withdrew from therapy without their doctors' consent. Follow-up included periodic testing of virologic, serologic and biochemical parameters for up to 5 years.**Results:** ALT normalization, serum HBV DNA undetectable, e-loss and e-conversion at 24 weeks were: 93.2 % (55/59), 79.7 % (47/59), and 79.6 % (47/59) respectively. The relapse rates in patients with HBeAg-positive, e-loss or e-conversion at the off-treatment time point were 90, 78.4, and 45.8 %, respectively at 6-month follow-up. Patients with HBeAg seroconversion show a lower recurrence rate with longer consolidation treatment (consolidation treatment more than 24 months, 16.7 %; consolidation treatment between 12 and 24 months, 42.8 %; consolidation treatment under 12 months, 100 %).**Conclusions:** HBeAg-positive patients being considered for withdrawal from NUC treatment should meet the following criteria: achieve HBeAg serological conversion, non-detectable HBV DNA and alanine aminotransferase normalization. Furthermore, the longer the consolidation treatment, the lower the recurrence rate. More than 2-years of consolidation therapy is recommended. Patients should be closely followed after off-treatment and avoid premature therapy withdrawal.*P-0483***Functional cure of HBV in a severe immunocompromised patient with CLL treated with Entecavir****Antonio Izzi¹, Antonio Ascione², Vincenzo Messina³, Basilio Fimiani⁴**¹Department of Infectious Diseases and Emergency Infectious Diseases D. Cotugno Hospital Naples, Italy; ²Fatebenefratelli Hospital Liver Unit Naples Italy; ³AO S. Anna e S. Sebastiano Infectious Diseases Unit Caserta Italy, Caserta, China; ⁴Umberto 1 Hospital Internal Medicine Unit, Nocera Inferiore, Italy

Background: Reactivation of an occult HBV infection (OBI) is frequent during polichemotherapy regimens including rituximab (RTX). Entecavir (ETV) treatment after a de novo hepatitis B following rituximab for Lymphoproliferative Disorders CD20+ is safe and effective.

Case Report: On October 2009, a 65 years-old patient, male, Caucasian, experienced a reactivation of OBI following a polichemotherapy with RTX-containing regimen due to a CD20+ Chronic Lymphocytic Leukemia (CLL).

Basal features: ALT levels 10 times \times ULN, HBsAg positive, Anti-HBs negative, HBeAg positive, Anti-HBc-IgM negative, HBV-DNA over 100 million IU/ml. ETV 0.5 mg/daily was promptly started.

Results: ALT became normal within 2 months, while HBV-DNA was undetectable in RT-PCR at the end of first year of treatment. At same time HBsAg became negative. Three years later, in November 2014, patient seroconverted to Anti-HBs more than 100 mIU/ml, confirmed in October 2015. CLL evolved towards a Diffuse Large B Cells Lymphoma (DLBCL, Richter Syndrome) despite persistent antineoplastic therapy with citarabine and RTX (ARAC-RTX).

Conclusions: This case report reveals the great effectiveness of ETV, not only for suppressive HBV therapy, but also to induce HBsAg loss and anti-HBs seroconversion (functional cure) in a severe immunocompromised patient undergoing a long-term treatment with an anti-CD20 MAB as RTX.

P-0484

Testing of kidney transplantation candidates for HBV infection and the importance of monitoring

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Objective: Testing for the markers of hepatitis B virus infection and prophylactic administration of antiviral agents in HBV-infected and HBsAg-positive individuals must not be neglected in kidney transplantation candidates due to the administration of immunosuppressive therapy. The present study aimed to evaluating the data of an Education Hospital on kidney transplantation in the last 12 years.

Method: The records of kidney transplantation recipients and their donors that underwent kidney transplantation at Organ Transplantation unit of Izmir Bozyaka Education and Research Hospital between 2003 and 2015 were retrospectively evaluated.

Results: The data of a total of 362 cases operated in the specified 12-year period were evaluated. HBsAg was positive in four cases and 53 cases tested positive for antiHBcIgG and antiHBs, and 8 cases tested positive only for antiHBcIgG. Antiviral prophylaxis was administered to all HBsAg positive cases.

Conclusion: In long-term follow-up, none of the operated cases showed HBV reactivation. Due to the fact that immunosuppressive therapy causes HBV reactivation in both HBsAg-positive cases and in those that developed immunity against the infection (antiHBcIgG and antiHBs-positive cases), all kidney transplantation candidates must be evaluated for HBV infection status and those testing positive for the HBsAg positive should receive antiviral prophylaxis.

P-0485

Network meta-analysis of nucleotide analogues for preventing HBV reactivation during chemotherapy

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Background: Five nucleotide analogues (NAs), including lamivudine, entecavir, adefovir, telbivudine and tenofovir are available to prevent chemotherapy-induced HBV reactivation. However, the optimum regimen has not been determined. Here we compare efficacy of these prophylactic agents in patients with chronic hepatitis B virus (HBV) infection (CHB) during the chemotherapy in terms of HBV reactivation and HBV-related complications.

Methods: PubMed, Embase and Cochrane Library database were searched in English and Chinese up to March 2015. Clinical trials and cohort studies of above five interventions in CHB patients undergoing chemotherapy were included. Network meta-analysis combined direct and indirect evidence to estimate. Odds ratios (ORs) for each clinical outcomes and the probabilities of ranking for optimal treatment were calculated in the network meta-analysis.

Results: Fifty-two eligible articles consisting of 3894 participants were included. For HBV reactivation, prophylactic treatment with NAs were all significantly superior to no prophylaxis, with OR from 0.00 [95 % credible interval (CI) 0.00–0.04] for the most effective intervention (tenofovir) to 0.10 (CI 0.06–0.14) for the least effective intervention (lamivudine). For HBV-related complications, prophylactic treatment with NAs also significantly outperformed no prophylaxis. Entecavir was most efficacious in reducing the risk of HBV related hepatitis (predicted probability, 83 %), HBV related death (68 %) and all causes of hepatitis (97 %). It ranked second in decreasing overall incidence of death (34 %).

Conclusion: Available evidence supports prophylactic therapy with tenofovir and entecavir as the most potent interventions in prevention of HBV reactivation and HBV-related morbidity and mortality for CHB infection patients undergoing chemotherapy.

P-0486

Follow-up of the chronic HBV infected patients planned chemotherapy due to solid organ malignancy

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The objective of this study was to screen the patients with solid organ malignancy for HBV infection before the start of chemotherapy in the oncology department of our setting. Our additional purposes were to define the risk groups for HBV reactivation and thereafter starting lamivudine prophylaxis to eligible patients to prevent from HBV reactivation and hepatitis. All cases admitted to oncology department for chemotherapy were screened prospectively for HBV between March 2013–September 2014. Two hundred and twenty five patients were included in the study. A total of 118 had no HBV infection and 34 were immunized. Others were divided into 3 groups; Group I: having recovered past HBV infection: 43 patients (19.1 %), Group II: isolated Anti-HBc total positive: 20 patients (8.9 %) and Group III: chronic HBV infection with 10 patients (4.4 %). In group I, there was no HBV reactivation and hepatitis. In group II, HBV reactivation developed in 1 (5.9 %) of 17 patients. In group II, there was occult HBV infection in 1 (5.9 %) of 17 patients. Lamivudine prophylaxis was started to these two patients. In group III, three were already receiving antivirals, lamivudine prophylaxis was started in 7 patients before their chemotherapy regimen. While under lamivudine prophylaxis 2 (28.6 %) of 7 patients developed HBV reactivation. During the follow up neither hepatitis by reactivation of HBV nor HBV related death were observed in our study. In conclusion, in the moderate endemicity areas like our country for HBV infection, all patients must be screened for HBV before the starting of chemotherapy.

P-0487

Entecavir to prevent HBV reactivation by chemotherapy for CHB patients with non-hematological cancer

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Background: Reactivation of HBV occurred in 20–50 % of CHB carriers undergoing cancer chemotherapy and might cause liver failure and death. Limited data on the efficacy of entecavir, antiviral with high potency and low resistance rate, to prevent HBV reactivation induced by chemotherapy for CHB with non-hematological malignancies.

Patients: Beginning from July 2010, 117 consecutive CHB carriers who treated with prophylactic entecavir at the onset of cancer chemotherapy were enrolled. Patient composition was as follows: breast cancer (39.3 %), lung cancer (17.9 %), colon cancer (17.1 %), head and neck cancer (10.3 %), other gastrointestinal tract malignancies (6.8 %), gynecological cancer (4.3 %) and others (4.3 %).

Results: Mean age of enrolled patients was 55.1 ± 1.0 years, 66 were male, and 25.6 % with baseline abnormal ALT. The mean baseline HBV DNA was 3.27 ± 0.14 log₁₀ IU/mL and 58 (49.6 %) of them >2000 IU/mL. The median duration of entecavir was 14 months (range 6–60). For baseline HBV DNA >2000 IU/mL group, undetectable HBV DNA (<20 IU/mL) at month 6, 12, 24 and 36 were 86.2, 92.7, 90.0 and 92.9 %, respectively. The undetectable HBV DNA rates at month 6, 12 and 24 were 100, 100 and 100 %, respectively for HBV DNA <2000 IU/mL group. No phenomenon of HBV reactivation was found and one patient had virological breakthrough due to non-compliance. Grade II hepatitis (ALT >2 × ULN) occurred in 30.8 % of patients but none could attributed to HBV reactivation.

Conclusions: Entecavir is highly effective to prevent HBV reactivation by chemotherapy for CHB patients with non-hematological malignancies.

P-0488

Cessation of pre-emptive therapy for patients with rheumatic disease with previously resolved HBV

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Methods: We examined the rate of HBV DNA reappearance after the cessation of entecavir (ETV) for rheumatic disease (RD) patients with resolved hepatitis B virus (HBV) being treated with pre-emptive ETV. The criteria for cessation of ETV were as follows: (1) at least 6 months of administration of ETV; (2) undetectable HBV DNA; (3) negative HBeAg; (4) negative HBsAg; and (5) negative HBV core-related antigen levels. After cessation of ETV, HBV DNA were monitored on a monthly. The efficacy outcome was reappearance of HBV-DNA.

Results: All patients (rheumatoid arthritis; n = 3 and rheumatic polymyalgia rheumatic (PMR), were men with mean duration of ETV administration of 19.5 months. For case 1, after prednisolone had been tapered and discontinued, the five ETV discontinuation criteria were met and ETV was stopped. At 39 months after cessation of ETV, HBV DNA reappearance had not occurred. For case 2, HBV DNA reappearance developed once 8 months after cessation of ETV while the patient was being treated with dexamethasone palmitate and MTX; however, it was not seen in the next 9 months. For case 3, reappearance of HBV DNA was noted at 27 and 34 weeks after cessation of ETV while being treated with tocilizumab. HBV DNA became negative immediately thereafter at both time points without re-administration of ETV. For case 4, reappearance of HBV DNA has not developed since cessation of ETV.

Conclusion: Our criteria for cessation of ETV may be effective for protecting against hepatitis onset in RD patients with resolved HBV.

P-0489

The efficacy of warning popup system in electronic medical chart in screening for HBV reactivation

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Background: In Japan, Guideline for the prevention of HBV reactivation was proposed by the study group backed up from health and welfare authorities. However, testing HBV markers has not yet widespread. In order to promote screening for HBV reactivation, warning popup system (WPS) on electronic medical chart (EMC) was introduced in our institution.

Aim: The aim was to evaluate the effect of WPS for the screening.
Methods: Using a retrospective electronic chart review, we identified the frequency of HBV screening testing in accordance with guideline for patients before and after introduction of WPS.

Results: Chemo- or immune-suppressive therapy was given in 499 patients before introduction of WPS, and also in 257 after WPS. Before WPS, HBsAg was tested for 439 (88.0 %) patients and not detected in 422. After WPS, 252 (98.1 %) patients were tested, and 243 were negative for HBsAg. Among patients with HBsAg, 14 of 17 patients before WPS and 9 of 9 after WPS were tested for HBV DNA. For those without HBsAg, frequency of testing for HBcAb and HBsAb increased after WPS; 44.1 % (186/422) in pre- to 66.7 % (162/243) in post-WPS period. As for quantified HBV DNA in patients with HBcAb and/or HBsAb, 56 (65.9 %) out of 85 patients were tested before WPS, and 48 (66.7 %) of 72 after WPS. Achievement ratio for screening proposed by the Guideline had risen from 34.3 % before WPS to 67.7 % after WPS.

Conclusion: WPS in screening for HBV reactivation is effective.

P-0490

A prospective evaluation of occult HBV infection in immunized infants born to HBsAg-positive mothers

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Background: To prospectively evaluate the prevalence of OBI in immunized infants born to HBsAg-positive mothers by performing a three-year consecutive follow-up in a birth cohort.

Methods: Overall 158 successfully immunized infants born to treatment-naïve HBsAg-positive mothers were subjected to serum HBV DNA testing at 7 months. OBI infants were followed up at 12, 24 and 36 months for HBV serological markers and HBV DNA.

Results: At 7 months, OBI was found in 20.3 % (32/158) of the infants, overall 25.0 % (8/32), 25.0 % (7/28) and 9.5 % (2/21) of the OBI infants continued to be positive for serum HBV DNA at 12, 24 and 36 months, respectively. Serum HBV DNA was positive at least once in 40.6 % (13/32) of the OBI infants during follow-up and 18.8 % (6/32) experienced a reappearance of HBV DNA in serum. None of the OBI infants became HBsAg-positive during follow-up. Anti-HBc was found to be positive in 83.3 % (15/18), 14.3 % (4/28), 6.3 % (2/32), 4.0 % (1/25) at 7, 12, 24, 36 months, respectively. One anti-HBc-negative OBI infant at 12 months became anti-HBc-positive at 24 months and subsequently negative again at 36 months. A much lower proportion of OBI infants received the first dose within 6 h after birth than HBV DNA negative counterparts (59.4 vs. 82.9 %, $P = 0.004$).

Conclusion: The rate of OBI is about 20.3 % in this target group. Consecutive serum HBV DNA testing is a surrogate of cccDNA detection in OBI infants. Timely administration of first dose of vaccine might reduce the occurrence of OBI.

P-0491

In a patient who is HBsAg-negative and AntiHBs positive, reactivation of hepatitis B infection

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Case: A 61-year-old male patient autologous bone marrow transplantation 8 months ago with the diagnosis of multiple myeloma. Prior to transplantation, patient was HBsAg negative, anti HBs and anti HBcIg G positive and liver functions were normal. On the 8th month of bone marrow transplantation, patient referred to our clinic with complaints of loss of appetite, fatigue and icterus on skin. In physical examination, all skin and sclera was icteric, others system were normal. The results of the investigations were as follows: AST: 1216 U/L, ALT: 3013 U/L, total bilirubin: 9.32 mg/dl. Hepatitis marker investigation results were as follows HBsAg, Anti HBc IgM: Anti-HBcIg and Anti HBs: positive. Patient was admitted to department of infectious diseases. In follow up evaluations, AST regressed to : 282 U/L, ALT: 624 U/L. Total bilirubin value was : 14,39 mg/dl. Tenofovir disoproxyl fumarate 245 mg/day was commenced with the diagnosis of hepatitis B reactivation and supportive treatment was given. Symptoms regressed and patient was discharged eventually.

Discussion: In patients with hepatitis B surface antigen, hepatitis B infection may develop after bone marrow transplantation as a consequence of virus reactivation. However, the development of hepatitis after transplantation has rarely been reported in patients who are anti HBs positive and HBsAg negative prior to bone marrow transplantation (1–3). In the immunosuppressive period after bone marrow transplantation, hepatitis B virus which is latent in liver cells may proliferate. Patients on immunosuppressive treatment, should be closely and carefully monitored even if they have negative HBsAg and positive Anti-HBs.

P-0492

The value of HBV-PreS1 and other HBV antigens for hepatitis B virus associated glomerulonephritis

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Objective: To investigate the potential value of serum hepatitis B virus PreS1, HBeAg and HBV DNA as a new noninvasive diagnostic indicator for HBV-associated glomerulonephritis (HBV-GN).

Methods: A total of 239 patients including 76 with HBV-GN, 85 with non-HBV-GN, and 78 with chronic hepatitis B (CHB) without renal disease were examined for serum PreS1, serum HBV markers using enzyme-linked immunosorbent assays and serum HBV DNA levels using polymerase chain reaction (PCR).

Results: HBV-GN group, non-HBV-GN group, the positive rate of serum PreS1 76, 40 %, hepatitis B without renal impairment group

65.4 %; the positive rate of HBeAg 63.2, 36.8, 20.5 %; hepatitis B virus marker HBV DNA high copy number (copy number $>1 \times 10^5$ IU/ml) 69.7, 35.3, 30.9 %, the difference was significant statistical significance.

Conclusions: Nephritis associated with hepatitis B HBV DNA high copy rate, positive rate, positive rate of HBeAg and PreS1 suggested the possibility of a larger renal injury associated with hepatitis B.

Keywords: PreS1 antigen, HBV-DNA, HBV associated nephropathy, HBV-Ag.

P-0493

Expression and significance of HBeAg, HBV-DNA in the patients with HBV associated nephropathy

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Objective: In this study, the serum HBeAg, HBV-DNA and other antigens would be detected and the relationship between the HBV antigens and HBV associated nephropathy would be explored.

Methods: retrospectively collected 178 patients with hepatitis B and chronic renal injury as the experimental group, and 156 patients with hepatitis B without renal injury as the control group. Collected the clinical data, for age, gender, liver function index (ALT, PLT, total bilirubin, serum albumin), quantitative examination of 24 h urinary protein, creatinine, eGFR. And detected the HBV-DNA, HBeAg.

Results: The age, liver functional indicators, renal functional indicators, incidence of liver cirrhosis and serum albumin had significant differences. There were significant differences between the two groups of the data of the percentage of high HBV-DNA copy ($>1 \times 10^5$ IU/ml) ($P < 0.01$), the positive rates of HBeAg ($P < 0.01$).

Conclusion: The percentage of high HBV-DNA copy ($>1 \times 10^5$ IU/ml) and the positive rates of HBeAg is higher of patients with HBV-associated nephropathy; High percentage of high HBV-DNA copy and the positive rates of HBeAg could suggest the HBV-associated nephropathy; The serum albumin and the positive rates of HBeAg and high HBV-DNA copy could help diagnose the HBV associated nephropathy.

Keywords: HBeAg, HBV-DNA HBV associated nephropathy.

P-0494

Change in renal pathological of HBV-GN and NHBV-GN

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Objective: To compare pathologic changes of kidneys in hepatitis B virus associated nephritis and non-hepatitis B virus associated nephritis.

Methods: 161 patients with glomerulonephritic disease with hepatitis B virus infection were experienced biopsy. We examined renal tissues processed and stained by immunohistochemistry, HE, PAS, PASM and Masson, meanwhile serum HBsAg, Anti Change in renal pathological of HBV-GN and NHBV-GN HBs, HBeAg, Anti-HBe, Anti-HBc, HBV-DNA, and HBV-DNA in the renal tissues were assayed by PCR, Southern and molecular cross assay. According to the above results, patients with HBV-DNA positive would be assigned into HBV-GN group and the rest would be assigned into control group.

Results: 76 of 161 cases (47.2 %) were diagnosed as HBV-GN, of which 40 cases (52.63 %) as membranous nephropathy, 20 cases (26.32 %) as membranoproliferative glomerulonephritis, 9 cases (11.84 %) as IgA nephrosis, 7 cases (9.21 %) of focal segmental glomerulosclerosis (FSGS); 85 cases (52.79 %) were diagnosed as NHBV-GN, of which 35 cases (41.17 %) as IgA nephrosis, 17 cases (20 %) as membranous nephropathy, 14 cases (16.47 %) as membranoproliferative glomerulonephritis, 12 cases (14.11 %) as minimal change glomerulopathy, 7 cases (8.23 %) of focal segmental glomerulosclerosis (FSGS).

Conclusion: There are different pathologic types between HBV-GN and of NHBV-GN, membranous nephropathy (MN) was still the main pathologic type for HBV-GN, and for NHBV-GN, IgA nephrosis was the main type.

Keywords: HBV-GN; NHBV-GN; Renal biopsy.

P-0495

Lamivudine with adefovir dipivoxil therapy does not worsen renal function in chronic hepatitis B

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Background: The aim of this study was to compare renal dysfunction and hypophosphatemia in adefovir dipivoxil (ADF) plus lamivudine (LMV) therapy versus ADF therapy alone in chronic hepatitis B (CHB) patients.

Methods: The case reports of 56 patients treated with 10 mg/day ADF plus 100 mg LMV (Group A) and 41 patients treated with 10 mg/day ADF (Group B) between March 2005 and February 2014 were reviewed. We evaluated the effects of estimated glomerular filtration rate (eGFR), serum creatinine, and serum phosphate levels at the start of ADF plus LMV or ADF monotherapy and followed up every 3 months.

Results: The median treatment duration was 73.6 and 80.1 months in groups A and B, respectively. Increased creatinine level (> 0.3 mg/dl) was observed in seven patients in group A and one patient in Group B (12.3 vs. 2.4 %, $P = 0.134$). Decreased eGFR (>50 %) was observed in 3 patients in group A and no patients in group B (5.3 vs. 0 %, $P = 0.262$). Hypophosphatemia occurred in 11 (24.6 %) patients in Group A and 14 (26.8 %) patients in Group B ($P = 0.799$). Mean serum creatinine levels increased and mean eGFR decreased from baseline to end of treatment in Group A ($p < 0.01$). Mean serum creatinine levels and mean eGFR did not change from baseline to end of treatment in Group B ($P = 0.410$).

Conclusions: Neither long-term ADF plus LMV therapy nor ADF monotherapy substantially negatively affected renal function. However, a mild decrease in eGFR and an increase in serum creatinine were observed in ADF plus LMV therapy compared to ADF monotherapy.

P-0496

Comparison of Telbivudine and Entecavir on maintenance of eGFR in off-treatment after 3-years

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Background: Treat chronic hepatitis B (CHB) patients with telbivudine (LdT) has been associated with long-term and off treatment improvement in estimated glomerular filtration rate (eGFR).

Methods: CHB patients completed 3-years of therapy with LdT or ETV and 1-year follow-up without viral breakthrough were enrolled in this study. eGFR was recorded at baseline, at 3-years, and after 1 year off treatment by MDRD, eGFR increasing more than 10 % from baseline was identified as improved.

Results: Forty-six patients (mean age 46.3) and 46 patients (mean age 51.3) completed 3 years therapy with LdT and ETV, respectively. Their baseline demography and pretreated conditions were comparable. The eGFR changed from 94.3 ± 28.3 to 104.0 ± 31.2 at year 3 and to 104.0 ± 28.8 (mL/min/1.73 m²) at year 4 in LdT patients and changed from 93.1 ± 26.1 to 85.5 ± 25.1 at year 3 and to 87.7 ± 24.8 (mL/min/1.73 m²) at year 4 in ETV patients. At year 4, 50.0 % patients had an improved eGFR from baseline in LdT patients and 13.1 % in ETV patients. Multivariate analysis of patients' age, gender, pre-treatment eGFR, HBeAg status, HBV DNA levels and medication, the only predictor for off treatment eGFR improvement was LdT therapy [odds ratio (OR), 6.31 (2.07–19.23), $p = 0.001$].

Conclusions: The study reported selected CHB patients without viral breakthrough during on and off treatment. The benefit of LdT on eGFR improvement maintained after off treatment and it is unique between ETV because the effect was not related to viral suppression.

P-0497

Prevalence of chronic kidney disease in patients with chronic hepatitis B: a cross-sectional survey

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Background: We aimed to investigate the prevalence of chronic kidney disease (CKD) in patients with chronic hepatitis B (CHB) who are on antiviral therapy.

Methods: This is a cross-sectional study in a real-life cohort, in which all patients are receiving antiviral treatment, with age over 30-years-old. CKD was defined as estimated glomerular filtration rate (eGFR) less than 60 mL/min per 1.73 m² or the presence of albuminuria or biopsy-proven glomerulonephritis. EGFR was calculated by Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula, while presence of albuminuria was defined as urinary albumin to

creatinine ratio (ACR) ≥ 3 mg/mmol. Univariate and multivariate analysis were conducted to determine the risk factors of CKD.

Results: Of 1334 patients, the overall prevalence of chronic kidney disease was 7.5 %, in which 14 patients with eGFR less than 60 mL/min per 1.73 m², 91 patients with albuminuria and 4 patients with biopsy-proven glomerulonephritis, respectively. Compared with participants without indicators of kidney damage, patients with CKD were elder (45.38 ± 9.96 vs. 42.48 ± 8.48 , $p = 0.005$), had lower level of HBsAg [\log_{10} IU/ml, median (IQR); 2.57 (2.01–3.08) vs. 2.88 (2.44–3.25), $p = 0.001$] and higher prevalence of cirrhosis (35.0 vs. 20.7 %, $p = 0.001$). In multivariate analysis, hypertension [odds ratio (OR) = 4.968; 95 % CI 2.873–8.592, $p < 0.001$], diabetes (OR = 4.222; 95 % CI 2.254–7.907, $p < 0.001$), and level of HBsAg (OR = 0.777; 95 % CI 0.612–0.987, $P = 0.038$) were independent factors associated with the presence of CKD.

Conclusion: CKD has become an important problem in patients with CHB. Special attention should be paid to patients with hypertension, diabetes and lower level of HBsAg.

P-0498

Prevalence of hepatitis B virus serologic markers in pregnant patients in a state hospital in Turkey

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Background and objectives: Hepatitis B virus (HBV) infection is a major public health problem in Turkey. Its severity is related to the risk of chronicity, especially in case of neonatal contamination. Our objectives were to investigate the prevalence of HBV infection among pregnant patients at the Zubeyde Hanim obstetrics and gynecology hospital by detecting HBsAg and to evaluate the risk of HBV mother to child transmission by screening for HBeAg.

Methods: We conducted a 19 months prospective study from February 2014 to August 2015. All pregnant patients consulting for antenatal care were screened for HBV serologic markers.

Results: The prevalence of HBsAg was 3.8 % (40 out 1050 screened patients). The average age was 24.51 years (17, 35 years). Most patients tested were unaware of their hepatitis B status and only 1.8 % had been vaccinated before pregnancy. Only 3 (7.5 %) of the 40 patients with HBsAg was positive for HBeAg.

Conclusion: Hepatitis B is very frequent in pregnant patients in Turkey and it is recommended that all pregnant patients be routinely screened for HBsAg. This screening of maternal infection would allow applying prophylactic measures to neonates to decrease the risk of disease chronicity.

P-0499

How does maternal hepatitis B surface antigen carrier status impact the preterm delivery?

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Objective: The aim of this study was to examine the impact of maternal hepatitis B surface antigen (HBsAg) carrier status on pregnancy-related complications including gestational diabetes

mellitus (GDM), preterm birth (PTB), intrauterine growth restriction (IUGR).

Methods: We analyzed pregnancy-related complications, outcomes and fetal growth index in 140 HBsAg positive pregnant women during the period of 2011–2015, with 155 HBsAg-negative pregnant women in the same period serving as controls. Logistic regression was used to examine the association between maternal hepatitis B surface antigen (HBsAg) carrier status and pregnancy-related complications.

Results: The HBsAg-positive pregnant women showed a significantly higher incidence of preterm delivery than the control group (3.54 vs 0.3 %, $P = 0.02$). The incidence of IUGR was also significantly higher in HBsAg-positive group (9.23 vs 2.02 %, $P = 0.03$). The incidence of GDM showed no significant difference between the two groups ($P > 0.05$). HBsAg positivity and abnormal ALT were determined as the risk factors for preterm delivery.

Conclusion: Women with hepatitis have an increased risk for complications during pregnancy. HBsAg carrier status can increase the risk of preterm delivery and IUGR in pregnancy.

P-0500

Tenofovir treatment for a Japanese female with chronic hepatitis B and pregnancy

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Oral nucleoside/nucleotide analogues (NAs) are the effective drugs for chronic hepatitis B virus (HBV) infection. The effect of NA such as tenofovir dipivoxil (TDF) on pregnancy seems minimal. We experienced a 37-year-old HBeAg-positive female with chronic HBV genotype C infection who has been treated with TDF and has normal pregnancy and delivery. She previously had standard interferon-alpha, lamivudine (LAM) plus adefovir (ADF), or peginterferon. Although she took LAM plus ADF and had no NA-resistance-mutations of LAM, ADF, ETV or TDF, HBV DNA did not become negative. After 3 months of ETV plus ADF treatment, her HBV DNA became below 2.1 LC/mL. As she wanted her baby, ETV plus ADF was stopped and TDF (300 mg daily) was started. After 4 months of commencement of TDF, her HBV DNA became negative and she got pregnant. After 13 months of commencement of TDF, She had a baby and her liver function remained normal. Although we experienced chronic HBV-patients treated with TDF, who had pregnancy and normal delivery, careful attention and follow-up will be needed in the treatment course of TDF for HBV infection and pregnancy.

P-0501

Hepatitis B virus in pregnancy: antiviral treatment efficacy and risk of perinatal transmission

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Objectives: To evaluate the efficacy of antiviral therapy and immunoprophylaxis to prevent potential risk of mother-to-child/vertical HBV transmission.

Methods: Data of 108 HBsAg positive pregnant women and their children at 10 referral hospitals were included.

Results: Twenty-six (24.1 %) pregnant women were HBeAg-positive, mean HBV-DNA levels at 1st, 2nd, and 3rd trimesters were 2.5×10^6 , 106.3×10^6 , 20.5×10^6 IU/ml, respectively. Twelve (11.1 %, only 3 HBeAg-positive) already had a total of 15 HBsAg positive children (3 had received both HBV vaccine and HBIG, 7 only HBV vaccine, 5 none) priorly. Three of their older children had CHB. Four (33.3 %) out of 12 infants with at least one HBsAg-positive sibling developed HBsAg-positivity despite active-and-passive immunoprophylaxis. Only one had HBeAg-positive mother, and only two mothers had HBV-DNA levels $>200,000$ IU/ml at 3rd trimester. Twenty-one pregnant women received antiviral treatment at a mean gestational age of 23.3 (12–32) weeks to treat CHB and/or to decrease HBV-DNA levels to prevent vertical transmission [16 (76.2 %) tenofovir, 3 (14.3 %) telbivudine, 2 (9.5 %) lamivudin]. None of their infants were HBsAg-positive or had any birth defects, and all had protective levels of anti-HBs. Totally, five (4.6 %) infants were HBsAg-positive despite active and passive immunization, four (80 %) had at least one HBsAg-positive sibling and were breast-fed. Only two (40 %) had HBeAg-positive mothers with HBV-DNA levels more than 200,000 IU/ml.

Conclusions: HBeAg-positive pregnant women with high HBV-DNA levels ($>200,000$ IU/ml) should receive antiviral therapy at 3rd trimester of pregnancy to prevent HBV transmission. Presence of a HBsAg-positive sibling may play a role in vertical HBV transmission, despite active and passive immunization, regardless of HBeAg status and HBV-DNA level of the mother.

P-0502

Diagnostic utility of AFP in hepatitis B related hepatocellular carcinoma

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Background: AFP is onco-development protein and designated as tumor marker such as hepatocellular carcinoma (HCC). HCC is the third most cause of cancer death worldwide. In Bangladesh hepatitis B causes more than 61 % of HCC and HBsAg positivity in the healthy population is 5.4 %.

Objectives: Find out the diagnostic utility of AFP in hepatitis B related HCC. **Methods:** This study was carried out in the department of Hepatology, BSMMU and approved by the Ethical Institutional Review Board (IRB). The diagnosis of HCC was confirmed by pathological examination or AFP elevation (400 ng/ml) combined imaging (CT/MRI) after exclusion of hepatitis C virus infection and significant alcohol intake. All patients were HBsAg positive by ELISA test.

Results: A total 44 patients were included in this study. Among them, 91 % were male (n = 40) and 09 % were female (n = 4). The mean age was 48.2 (12.9) years with range from 23 to 80. Cirrhosis was 79.5 % (n = 35) and no cirrhosis was found 20.5 % (n = 9). The difference was statistically significant (P = 0.001). AFP was elevated (400 ng/ml) 63.6 % (n = 28) and normal (15 ng/ml) 18.2 % (n = 8). The difference was statistically significant (P = 0.049). Among cirrhosis group AFP elevated in Child Pugh A, B and C was 3.6 % (n = 01), 35.7 % (n = 10) and 50.9 % (n = 14) respectively whereas in non-cirrhotic group 44.4 % (n = 04) shows normal AFP (P = 0.029).

Conclusions: From our observation we concluded that AFP is a significant marker for HCC but a larger portion having normal AFP. AFP increase is more in cirrhotic group.

P-0503

Reversible pSmad3 signaling between tumor suppression and fibrocarcinogenesis in chronic hepatitis B

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Objective: Nucleoside analogue treatment to chronic hepatitis B patients can improve hepatic fibrosis and reduce incidence of HCC. Insight into the hepatocytic fibro-carcinogenesis caused by HBV infection has come from recent analyses of TGF- β signaling. TGF- β type I receptor and c-Jun N-terminal kinases differentially phosphorylate a mediator Smad3 to create 2 isoforms phosphorylated (p) at the COOH-terminus (C) or at the linker region (L). This study aimed to elucidate how HBV infection affected hepatocytic Smad3 phosphoisoform signaling.

Methods: To clarify the relationship between Smad3 phosphorylation and liver disease progression, we studied 10 random patients in each stage of HBV-related liver disease (F1 to 4) and also 10 patients with HBV-associated HCC. We chose 27 patients with chronic hepatitis B who underwent baseline and follow-up biopsies at 52 weeks from the start of nucleoside analogue treatments. Fibrosis stage, inflammatory activity, and pSmad3/c-Myc positivity in the paired biopsy samples were compared.

Results: Hepatocytic tumor-suppressive pSmad3C signaling shifted to fibro-carcinogenic pSmad3L signaling as the livers progressed from chronic hepatitis B to HCC. Owing to nucleoside analogue treatment, serum ALT and HBV-DNA levels were dramatically decreased. As a result, 25 of 27 (93 %) patients displayed fibrosis regression and inflammation reduction in follow-up biopsy specimens. Likewise, decrease in HBV-DNA restored tumor-suppressive pSmad3C signaling in hepatocytes, while eliminating prior fibro-carcinogenic pSmad3L signaling and concomitantly dropping in c-Myc expression.

Conclusions: In chronic hepatitis B, oral nucleoside analogue therapies can regress fibrosis and reduce HCC incidence by successfully reversing pSmad3 signal from fibro-carcinogenesis to tumor-suppression.

P-0504

Partial virological response with entecavir is not risk factor of HCC occurrence

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Background: The aim of this study is to investigate whether partial virological response (PVR) to entecavir (ETV) treatment might affect long term clinical outcome including hepatocellular carcinoma (HCC) or not.

Methods: Treatment-naive chronic hepatitis B (CHB) patients treated with ETV (0.5 mg/day) for at least 1 year were subsequently followed up with regular surveillance of HCC occurrence. PVR was defined as a decrease in HBV-DNA titer of more than 1 log 10 IU/mL, by real time-polymerase chain reaction, but with residual serum HBV-DNA, at week 48 of ETV therapy.

Results: A total of 538 CHB patients (354 males and 184 females) were followed up for median 59.7 months. At 48 weeks after ETV treatment, complete viral response (CVR, HBV-DNA <20 IU/mL) was achieved in 392 (72.9 %) patients and PVR in 138 (25.7 %) patients. In univariate analysis, old age, liver cirrhosis, and non-ALT normalization at week 48 were related with HCC occurrence (all P < 0.05), whereas PVR was not significantly related with HCC occurrence (both P > 0.05). In multivariate analysis, age and liver cirrhosis were selected as independent risk factors of HCC occurrence (both P < 0.001). In sub-group of compensated cirrhosis, PVR was also not significantly related with HCC occurrence (P = 0.805).

Conclusions: Patients with PVR did not have a higher risk of HCC occurrence than those with CVR, suggesting early rescue therapy for patient with PVR might not be urgent unless patients are at high risk of HCC development.

P-0505

Notch ligand DNA copy number variation is enhanced in HBV-related liver cancer

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Introduction: We previously reported genomic amplifications in liver cancer were associated with a specific expression profile in α -fetoprotein producing hepatoma cells and involve the Notch ligand gene, Jagged1. We assessed Notch signaling-related genomic changes in HBV-infected cells and in clinical samples of HBV-related liver cancer tissue.

Methods: Jagged1 copy number variations (CNVs) in liver cancer tissues after surgical resection were analyzed and compared the outcome in cases of HBV or HCV infection. We measured Jagged1 CNVs in HepG2 and HBV infected HepG2.2.15.7 cells. HBV cccDNA copy numbers were measured and compared prognosis in Jagged1 amplified liver cancer samples.

Results: Analysis of clinical samples showed HBV-related liver cancer cases had more high levels of Jagged1 genomic CNVs, which were associated with significantly poorer rates of survival ($p < 0.05$) compared with HCV-related liver cancer cases ($P = 0.68$). We observed significant Jagged1 genomic amplification in HepG2.2.15.7 cells compared with HepG2 cells, which suggested HBV infection caused further Notch signaling abnormalities. HBV cccDNA was detected in liver cancer tissues as well as the surrounding liver tissues. However, we found liver cancer tissues without HBV cccDNA. HBV cccDNA patterns in Notch-upregulated samples were associated with rather good prognosis after surgical resection, whereas the prognosis was poor in HBV cccDNA-absent cases, suggesting that the tumor cells could not control HBV virus life cycles, especially in highly malignant types of liver cancer.

Conclusion: Jagged1 DNA copy number variations promoted malignant characteristics in HBV-related liver cancer.

P-0506

WFA+-Mac-2 binding protein is a predicting biomarker for HCC in hepatitis B patients

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Background/aim: Nucleoside analogue (NA) can not prevent to develop HCC in some patients.

Objects: 124 patients with nucleoside analogue (entecavir or tenofovir) therapy who have good efficacy (undetectable HBV-DNA) for more than 1 year (sex: M/F 70/54, age: 48.8 ± 10.7 , genotype: A/B/C/E/UD 1/10/101/1/11, HBsAg $\pm 67/57$, HBeAb $\pm 73/51$, HBV DNA

6.9 ± 1.7 log copies/ml, HBcrAg 5.7 ± 1.8 log U/ml, HBsAg 2735 IU/ml (3.4–207,888 IU/ml), were enrolled.

Methods: During 44 (12–147) months observation period, 13 of 124 patients developed HCC. In the two groups (HCC or non-HCC), we compared several factors including a novel glycomarker, WFA+-Mac-2 binding protein (M2BP) associated with liver fibrosis by univariate analysis, Kaplan–Meier method and Cox proportional hazards regression analysis search for predicting factors for HCC development during NA therapy.

Results: Kaplan–Meier analysis showed platelet count $< 12.2 \times 10^4/\mu\text{L}$, Fib-4 index > 3.52 and AFP > 8.6 ng/ml at baseline, and platelet count $< 8.9 \times 10^4/\mu\text{L}$, Fib-4 index > 3.0 , AFP > 4.0 ng/ml and WFA + - M2BP > 1.43 one year after NA therapy were significantly predictive factors for HCC development ($p < 0.0001$, $P = 0.0061$, $P = 0.0093$, $p < 0.0001$, $p < 0.0001$, $P = 0.0047$, $P = 0.0004$, respectively). Additionally, Cox proportional hazards regression analysis showed platelet count $< 12.2 \times 10^4/\mu\text{L}$ at baseline, as well as Fib-4 index > 3.0 , AFP > 4.0 ng/ml and WFA + - M2BP > 1.43 one year after NA therapy were independent risk factors for HCC development (OR = 5.7; $P = 0.0164$, OR = 9.3; $P = 0.0023$, OR = 4.8; $P = 0.00283$, OR = 7.9; $P = 0.0047$, respectively).

Conclusions: Among patients with NA therapy, lower platelet count at baseline, as well as higher Fib-4 index, AFP and WFA+-M2BP one year after NA therapy were independent risk factors for HCC development.

P-0507

IP-10, p53 and Foxp3 expression in hepatitis B patients with cirrhosis and hepatocellular carcinoma

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Elucidating differences in gene expression may be useful in understanding the molecular pathogenesis and for developing specific markers for the outcome of Hepatitis B virus infection. IP-10, p53 and Foxp3 were studied in liver cells to investigate a possible link between selected host gene expression and outcome of viral hepatitis B infection. The study was conducted on a total of 60 purposively selected patients who were divided into four groups, 15 in each including HBV positive cirrhosis, HBV negative cirrhosis, HBV positive HCC and HBV negative HCC. Total mRNA extraction was done from FNAC of liver followed by cDNA synthesis, and finally gene expression was analyzed using real time PCR technique. IP-10 and p53 gene expressions were lower in HBV positive cirrhosis except Foxp3 gene, which were up regulated in HBV positive cirrhosis in comparison to HBV negative cirrhosis. The expression of all the three genes among HBV positive HCC were up regulated in comparison to HBV negative HCC. Among HBV positive cirrhosis and HCC, the expression of IP-10, p53 and Foxp3 genes studied were up regulated in HBV positive HCC in comparison to HBV positive cirrhosis. In conclusion, there were variations in the expression of the genes among cirrhosis and HCC patients. All the three selected genes were more or less up regulated in HBV positive HCC patients. These three particular genes may be responsible for the molecular pathogenesis and clinical outcome of HBV positive patients. Thus, IP-10, p53 and Foxp3 genes may be used as biomarkers for HCC.

P-0508

Elevated aflatoxin B₁-albumin adducts increase cirrhosis and cirrhotic hepatocellular carcinoma risk**Yu-Ju Chu¹, Hwai-I Yang², Jessica Liu², Hui-Chen Wu³, Li-Yu Wang⁴, Sheng-Nan Lu⁵, Mei-Hsuan Lee⁶, Chin-Lan Jen², San-Lin You⁷, Regina M. Santella³, Chien-Jen Chen²**

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Background: Both aflatoxin exposure and chronic hepatitis B virus (HBV) infection synergistically increase the risk of hepatocellular carcinoma (HCC). Several important risk factors for HBV-related HCC were not considered in previous studies and the effect of aflatoxin exposure on cirrhosis remains unclear.

Method: A case-control study nested in the Community-Based Cancer Screening Project cohort was conducted to investigate the association between aflatoxin exposure and risk of cirrhosis and HCC after adjustment for important risk factors. Baseline serum levels of aflatoxin B₁ (AFB₁)-albumin adducts were tested for 232 cirrhosis, 154 cirrhotic HCC, 108 non-cirrhotic HCC cases, and 2527 unaffected controls. Logistic regression analyses were used to estimate multivariate-adjusted odds ratios (OR_a) with 95 % confidence interval (CI) for each risk factor.

Results: Among chronic HBV carriers in Taiwan, serum level of AFB₁-albumin adducts was associated with an increased risk of newly-developed cirrhosis and cirrhotic HCC in a dose-response manner (cirrhosis, *p*-trend = 0.0006; cirrhotic HCC, *p*-trend = 0.0001). No significant association with the serum level of AFB₁-albumin adducts was observed for non-cirrhotic HCC (*p*-trend = 0.1498). The OR_a (95 % CI) for detectable AFB₁-albumin adducts (vs. undetectable) was 1.8 (1.1–2.7) and 5.8 (1.3–25.0), respectively, for cirrhosis and cirrhotic HCC diagnosed within 7 years after enrollment; but not for cirrhosis or cirrhotic HCC diagnosed after 7 years of follow-up (cirrhosis, OR_a = 1.4, 95 % CI 0.5–3.8; cirrhotic HCC, OR_a = 0.8, 95 % CI 0.4–1.3).

Conclusion: Among chronic HBV carriers, aflatoxin exposure is significantly associated with an increased risk of cirrhosis and cirrhotic HCC in a time-dependent manner.

P-0509

Assessing HAV antibody in the Thai population**Pattaratida Sa-nguanmoo¹, Nawarat Posuwan¹, Preeyaporn Vichaiwattana¹, Viboonsak Vuthitanachot², Siriporn Sae-lao³, Monthana Foonoi³, Apinya Fakhongyoo³, Jamorn Makaroon⁴, Klaita Srisingh⁵, Duangporn Asawarachun⁶, Somchai Owatanapanich⁷, Norra Wutthirakowit⁸, Kraisoron Tohtubtiang⁹, Sompong Vongpunasawad¹, Yong Poovorawan¹**

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Although HAV is not endemic in Thailand, sporadic acute infections do occur. To determine the HAV seroprevalence in Thailand compared to data published in literatures since 1971, a total of 4260 individuals between 1 month and 71 years old from different geographical areas were screened for anti-HAV IgG antibody by chemiluminescent microparticle immunoassay. Overall frequency of detectable anti-HAV IgG antibody was 34.51 %. The prevalence rates were 27.26 % (North), 30.76 % (Central), 33.81 % (Northeast), and 45.82 % (South). Anti-HAV IgG seropositive correlated with increasing age, especially after the age of 30. Prevalence of HAV infection markedly declined in the younger age groups compared to previous studies from 1971 to 2004. The mean age of the population possessing fifty percent anti-HAV IgG antibody between 1971 and now demonstrated an increasing trend. The lower prevalence rate of HAV infection directly correlated with improved healthcare system and more developed economy over the past four decades. The available of an effective HAV vaccine may be beneficial to specific population groups to prevent HAV outbreak in Thailand.

P-0510

Corticosteroid therapy in relapsing hepatitis a: a case report**Ermil R. Magsino, Lord Byron C. Corral, Arlinking O. Go**

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Hepatitis A virus infection resolves in most patients uneventfully within weeks from the onset of the disease. Though it is usually a self-limiting disease, atypical and rare manifestations including prolonged cholestasis and relapsing course are well documented. Relapse is usually clinically milder than the first phase, with variable liver function abnormalities and a tendency toward more marked cholestatic features.

Case: A 26 year old male who developed jaundice, tea-colored urine and pruritus had markedly disturbed elevation of liver enzymes and bilirubins with concomitant reactive HAV antibodies was managed as a case of uncomplicated hepatitis A. The patient went spontaneously into clinical remission after 3 weeks with partial resolution of biochemical parameters. Unfortunately, symptoms reappeared a week after remission with further re-elevation of transaminases and bilirubins and persistent HAV antibodies.

Diagnosis/management: Relapsing hepatitis A infection is characterized by the biphasic peak of serum transaminase levels with worsening of clinical symptoms. HAV antibodies were also still detected even after the biphasic peak. Steroid treatment was eventually started and resulted in marked clinical and biochemical improvement.

Recommendation: In symptomatic relapsing hepatitis A patients, favorable response may be achieved with steroid therapy.

P-0511

Acute hepatitis A may trigger development of autoimmune hepatitis**Resat Ozaras¹, Veysel Tahan², Fehmi Tabak¹**¹Infection Department, Cerrahpasa Medical School, Istanbul University, Istanbul, Turkey; ²Gastroenterology Department, Missouri University, Columbia, MO, USA

High rates of autoantibodies in patients with acute hepatitis A virus (HAV) infection have been reported. Autoimmune hepatitis (AIH) is a chronic liver disease of unknown etiology characterized by hyperglobulinemia, the appearance of certain circulating autoantibodies, and inflammatory changes on liver histology. AIH may be triggered by many factors including drugs and viral hepatitis in predisposed individuals. In the first study, Vento et al. (Lancet 1991;337:1183) have monitored 58 relatives of 13 patients with AIH prospectively for 4 years. Subclinical acute HAV infection developed in three patients, two of whom developed type 1 AIH within 5 months. They described an intrinsic defect in suppressor-inducer T cells mediating immune reactivity to a liver antigen (asialoglycoprotein receptor-ASPGR; located on the hepatocyte cell surface) in these patients. In English literature, 9 more cases of AIH including our case report and a case of overlap syndrome (AIH/PBS) developed after acute HAV infection have been published. Beside, a case of AIH after HAV vaccination and two overlap syndromes after combined vaccine of HAV and HBV (one AIH/PBS and one AIH/PSC) were reported. All the clinical data and the results of the studies reporting high rates of autoantibodies in HAV infected patients support an association of AIH and acute HAV infection.

P-0512

Asymptomatic hepatitis E viremia in blood donors in Nepal**Ananta Shrestha¹, Birendra Gupta², Thupten Lama¹, Sandip Khadka¹**¹Liver Foundation Nepal, Kathmandu, Nepal; ²Central Department of Biotechnology, Tribhuvan University, Kirtipur, Nepal

Introduction: Hepatitis E Virus (HEV) has caused four large epidemics of acute hepatitis in Kathmandu Valley. In between epidemics, sporadic HEV cases accounts for 15–30 % of acute hepatitis. HEV viremia in healthy blood donor is increasingly recognised and is a significant threat for transfusion related hepatitis E.

Patients and methods: Blood samples were collected from 581 healthy blood donors from Kathmandu in February 2014. IgG Anti HEV, IgM Anti HEV, HEV Ag were assayed using ELISA (Wantai). Real time PCR was done on HEV Ag positive samples to detect HEV RNA.

Results: The study subjects comprised 401 men and 180 women volunteer blood donors with median age of 35 years. None of the subjects had elevated ALT greater than 2 times ULN. IgG anti-HEV was detected in 49 samples (8.4 %) and IgM anti-HEV was detected in 17 samples (2.9 %). HEV Ag was detected in 11 samples and HEV RNA was detected in 9 (1.56 %) of the blood donors. All HEV isolates were found to be of Genotype 1a.

Discussion: Detection of HEV RNA among healthy blood donors without any clinical, biochemical and serological features of HEV infection in a significant number of blood donors indicate an unusual

phenomenon. While its relation with clinical hepatitis is not well understood, it indicates potential threat of transfusion associated HEV infection. Further, these transiently viremic individuals may maintain HEV in the community and act like dynamic reservoirs.

P-0513

Chronic genotype 1 HEV infection in an immunosuppressed liver-transplant recipient in India**Sahaj Rathi¹, Manu Mehta¹, Mini P Singh¹, Sunil Taneja¹, Ajay Duseja¹, Ashim Das¹, R K Ratho¹, Rakesh Aggarwal², R K Dhiman¹, Y K Chawla¹**¹Department of Hepatology, Post Graduate Institute of Medical Education and Research, Chandigarh, India; ²Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow, India

Background: In recent years, several cases with chronic hepatitis E (CHE) have been reported among immunocompromised patients from developed countries. All such cases have been reported by infection with Hepatitis E virus (HEV) genotype 3, except one case related to genotype 4. None have been reported by genotype 1 HEV, the most common genotype prevalent worldwide, particularly in developing countries. We report the first case of CHE related to genotype 1 HEV in a liver transplant recipient.

Case report: A 48-year-old man who underwent living-donor liver transplantation for cryptogenic cirrhosis, developed progressive cholestatic jaundice 3 months later. Examination revealed deep icterus. He had thrombocytopenia, and mild-moderate elevation of transaminases and alkaline phosphatase. Liver biopsy suggested acute rejection, however despite corticosteroid pulse treatment, serum bilirubin rose to 58 mg/dl. Serum creatinine raised to 2.3 mg/dL. Further workup revealed presence of IgM anti-HEV and HEV RNA (real-time TaqMan RT-PCR) in serum. Partial HEV RNA sequencing at two different laboratories showed that the isolate belonged to genotype 1. HBsAg, anti-HCV antibody and autoimmune markers tested negative. A liver biopsy at this stage showed chronic biliary ductopenia. Immunohistochemistry suggested presence of HEV RNA. Three weeks after reduction in immunosuppression and low-dose ribavirin (200 mg/day), serum bilirubin declined to 42 mg/dl and serum creatinine become normal, prompting an increase in ribavirin dose to 400 mg/day.

Conclusion: Our patient, a liver transplant recipient on immunosuppressive therapy, had CHE due to genotype 1 HEV. Relationship of HEV infection to chronic ductopenia is unclear

P-0514

Three novel amino acid substitutions in RNA dependent RNA polymerase accelerates HEV infection**Birendra Prasad Gupta^{1,2}, Thupten Lama², Sandip Khadka², Anurag Adhikari², Ananta Shrestha²**¹Central Department of Biotechnology, Tribhuvan University, Kathmandu, Nepal; ²Liver Foundation Nepal, Kathmandu, Nepal

Background: RNA dependent RNA polymerase (RdRp), encoded by Open Reading Frame 1 (ORF1), is responsible for synthesizing complementary minus strand and subsequent synthesis of genomic

RNA, thus is of crucial importance in Hepatitis E virus (HEV) life cycle. Three mutations were detected in HEV isolates from an epidemic that took place in Biratnagar city of Nepal in 2014. The significance of these mutations is shown in present study.

Methods: HEV clone containing three non synonymous mutations inside RdRp viz; Y1358, A1404, E1491 (YAE) was constructed in pWPXL backbone. The replication kinetics was analyzed by polymerase activity whereas HepG2/C3A and Huh7 cell line were used to compare the infection severity of mutated HEV clones; and Balb/c nude mice was used to study expression of inflammatory cytokines.

Results: HEV clone containing YAE had 4.5 folds increment in RdRp polymerase activity in HepG2/C3A and 4 folds in Huh7 cell line compared to wild strain TK15/92 at 37 degree C. The TCID50 value was observed to be 10^{-7.5} and 10^{-7.4} in HepG2/C3A and Huh7 cell line, respectively, at 48 h post YAE infection. Expression of inflammatory cytokines KC, MCP-1, TNF- alpha, MIP-1alpha, RANTES and IP-10 was increased ($P < 0.001$) when mice were infected with YAE clone.

Conclusion: The adaptive changes in polymerase offered replication fitness to the virus. Inflammatory cytokines elaborated by the mutated strains were significantly higher as compared to wild strain of HEV. These mutations could be related to large epidemics and severe manifestations of HEV infection in humans.

P-0515

Sero-prevalence of acute hepatitis caused by HAV and HEV in Gazipur, Bangladesh

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Aim: In Bangladesh, poor sanitation and hygiene, lack of safe drinking water supply and overcrowding may be responsible for high prevalence of HAV and HEV infection in different age groups. This study was undertaken to determine the prevalence, age-specific prevalence and seasonal variation of HAV and HEV in Gazipur district of Bangladesh.

Methods: This cross-sectional study was conducted in 1118 patients presenting with acute viral hepatitis. All were tested for anti-HAV IgM and anti-HEV IgM by ELISA. The study population was divided into 7 groups according to their ages with 10 years interval.

Results: Among 1118 patients, 571 (51.0 %) and 203 (18.2 %) were found anti-HEV IgM and anti-HAV IgM positive respectively. Rest 344 (30.8 %) patients were negative for both anti-HAV IgM and anti-HEV IgM. 64.0 and 77.8 % male patients were positive for HAV and HEV. In our study, it was found that 62.4 % patients positive for HAV were <10 years and 73.9 and 70.5 % HEV positive patients were in 31–40 years and 41–50 years age group.

Conclusions: It may be concluded with that both HAV & HEV can occur at any age throughout the year, but HAV affects children more and HEV is common in adults with a peak during hot seasons with heavy rainfall. Ensuring supply of safe drinking water, proper sanitation, improvement of personal hygiene and most importantly raised public awareness remain important guards against these viruses for the time being.

P-0516

Is HEV screening necessary in patients of cirrhosis of Liver?

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Background: Recent studies on high anti-HEV (Hepatitis E virus) IgG rate in autoimmune hepatitis patients suggest HEV triggers the immune events. Data on seroprevalence of HEV infection in cirrhosis patients is limited. The study aimed to assess the magnitude of HEV infection in cirrhosis patients presenting with decompensation.

Methods: We performed a cross sectional study in which serological analysis was determined by anti HEV IgG ELISA (Diapro, SRL, Italy) according to the manufacturer's instructions. HEV RNA was detected by Viral RNA Extraction kit (Qiagen, Germany). Viral load was detected by HEV Real Time PCR kit. The possible association of anti HEV IgG and demographic characteristics of the patients were analyzed by univariate analysis.

Results: The study included 80 patients, which included 70 decompensated cirrhosis cases admitted in the medical wards of LNJP hospital, New Delhi and 10 healthy controls. The mean age of the cirrhotic patients was 44.27 years (20–75 years). HEV IgG was present in 81.42 % (57/70) liver cirrhosis cases. HEV superinfection was present in 33.3 % (4/12) of HCV related cases, 60 % (3/5) HBV related cases. HEV RNA was detected in 32.85 % (23/70) of decompensated cirrhosis patients. The mean HEV viral load in the cirrhosis patients was 10453.20 copies/ml.

Conclusions: The data indicate the high HEV seropositivity in patients with cirrhosis and HEV seroprevalence increases significantly with age. Further investigations are required to ascertain whether patients of decompensated cirrhosis in India need HEV screening prior to liver transplantation as there is likelihood of the graft getting infected.

P-0517

Epigenome deregulation in liver carcinoma

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Epigenetic mechanisms maintain heritable changes in gene expression and chromatin organization over many cell generations. Importantly, deregulated epigenetic mechanisms play a key role in a wide range of human malignancies, including liver cancer. Various environmental agents and lifestyles known to be risk factors for HCC (such as infection by hepatitis B virus (HBV) and hepatitis C virus (HCV), chronic alcohol intake, and aflatoxins) are suspected to promote its development by eliciting epigenetic changes, however the precise gene targets and underlying mechanisms have not been elucidated. Many recent studies have exploited conceptual and technological advances in epigenetics and epigenomics to investigate the role of epigenetic events induced by risk factors in liver tumors and non-tumor precancerous lesions. Our recent studies have identified a large number of genes and pathways that are targeted by epigenetic deregulation (changes in DNA methylation, histone modifications and RNA-mediated gene silencing) during the development

and progression of liver cancer. In addition, several lines of evidence argue that risk factors (such as HBV virus) may abrogate cellular defense systems, induce silencing of host genes and promote liver cancer development via an “epigenetic strategy”.

P-0518

Deep-sequencing reveals clonal evolution and genomic heterogeneity in multifocal liver cancer

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Aims: Multifocal tumors are very common in primary liver cancer. Whether they develop as independent tumors or as intrahepatic metastases are of clinical and theoretical importance.

Methods: A patient with synchronous two hepatocellular carcinoma (HCC-A and HCC-B) and one intrahepatic cholangiocarcinoma (ICC), as well as two postoperative recurrent tumors, was enrolled. Multiregional whole-exome sequencing was applied on these tumors to delineate the clonality and heterogeneity.

Results: Based on exome sequencing, 3 primary tumors showed almost no overlaps in mutations and CNV, suggesting that they developed through highly different genetic alterations. Within each tumor, multiregional sequencing data showed varied intratumoral heterogeneity (21.6 % in HCC-A, 20.4 % in HCC-B, 53.2 % in ICC), highlighting the limitations of single biopsy. The mutational profile of two recurrent tumors showed obvious similarity with HCC-A (86.7 and 86.6 % respectively), rather than HCC-B and ICC, indicating that they originated from HCC-A. The evolutionary history of the two recurrent tumors indicated that intrahepatic micro-metastasis could be an early event during HCC progression. Notably, FAT4 was the only gene that mutated in two primary HCCs and the recurrences but in different locations. Mutation prevalence screen and functional experiments clearly showed that FAT4, harboring somatic coding mutations in 26.7 % of HCC, could potently inhibit the growth and invasion of HCC cells. In HCC patients, both FAT4 expression and FAT4 mutational status significantly correlated with patient prognosis.

Conclusions: Spatial and temporal dissection of genomic alterations during the progression of liver cancer may help to elucidate the basis for its dismal prognosis.

P-0519

Genetic alterations of the SIAH-1 gene in hepatocellular carcinomas

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Background and aims: Siah-1 is the mammalian homolog of *Drosophila* seven in absentia (*sina*) and has been identified as a p53-

inducible gene. Siah-1 can induce cell cycle arrests, tumor suppression, and apoptosis through a novel beta-catenin degradation pathway. **Methods:** To determine whether genetic alterations of Siah-1 gene are involved in the development and/or progression of HCCs, we searched for mutation of the Siah-1 gene in 38 HCCs by single strand conformational polymorphism and sequencing. The effect of Siah-1 on beta-catenin degradation was further examined in wild- and mutant-type Siah-1 transfected HEK 293T cells.

Results: We found two frameshift mutations and one missense mutation of the Siah-1 gene. The cases with Siah-1 mutation showed nuclear translocation and cytoplasmic staining of beta-catenin. Interestingly, three mutants of Siah-1 stabilized cytoplasmic levels of beta-catenin, even after treatment of adriamycin. Furthermore, three mutants failed to suppress cyclin D1 expression and to induce apoptosis.

Conclusions: These data suggest that inactivating mutations of the Siah-1 may contribute to the development of HCCs through beta-catenin stabilization and apoptosis block.

P-0520

GENOMIC LANDSCAPE OF PRECANCEROUS LIVER DISEASE

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The development of hepatocellular carcinoma (HCC) is associated with genetic alterations and differential expression of genes involved in regulatory pathways. However, a unified mechanism of hepatocarcinogenesis has not been elucidated and there are clearly multiple HCC subtypes characterised by differing mutation profiles. We aimed to understand the mutational process involved in precancerous liver leading up to HCC.

We have performed whole exome sequencing (WES) of precancerous liver tissues: 18 patients including non-diseased donors ($n = 6$) and 12 HCV-positive patients with ($n = 6$) and without ($n = 6$) liver cirrhosis. We have also analysed non-diseased WES data from 1000 Genome Project and paired HCC and surrounding non-tumour liver samples (total of 178). To exclude germline mutations we filtered out mutations found in 1000 Genome and Allele Frequency Community databases.

In precancerous liver disease there were no recurrent mutations in driver genes associated with HCC nor significant pathway enrichment. However, there was a step-wise increase number of mutations from normal to HCC samples. Those mutations were evenly distributed across the genome and the majority was unique to the patient, suggesting they are passenger mutations.

Our results are consistent with differing genetic changes associated with HCC within the multiple datasets, and strongly suggest that mutation accumulation is a general mechanism in tumour evolution. Genomic alterations occur randomly in hepatocytes, independent of aetiological factors and accumulate leading to HCC. We are currently performing transcriptome analysis of sequenced samples to look at the correlation between genomic alteration and changes in the gene expression.

P-0521

Adenylate kinase 4 modulates drug sensitivity and mitochondrial activity**Koichi Fujisawa^{1,2}, Taro Takami², Naoki Yamamoto², Toshihiko Matsumoto², Takafumi Noma³, Shuji Terai⁴, Isao Sakaida^{1,2}**¹Center of research and education for regenerative medicine, Yamaguchi University School of Medicine, Japan; ²Department of Gastroenterology and Hepatology, Yamaguchi University Graduate School of Medicine, Japan; ³Department of Molecular Biology, Institute of Health Biosciences, The University of Tokushima Graduate, Tokushima, Japan; ⁴School Division of Gastroenterology and Hepatology, Graduate School of Medical and Dental Sciences, Niigata University, Japan

Adenylate kinase is a key enzyme in the high-energy phosphoryl transfer reaction in living cells. Of its isoforms, Adenylate kinase 4 (AK4) is localized in the mitochondrial matrix, and is believed to be involved in stress, drug resistance, the malignant transformation of cancer, and ATP regulation. It is reported that lung cancers with high AK4 expression showed increased malignancy. More recently, surprising result that AK4 was the key regulator of intracellular ATP levels was reported by screening of RNAi library. We evaluated the AK4 expressional level in various cell lines, and found that AK4 is induced by hypoxia and deferoxamine (DFO) in most cell lines including Hep3B (hepatoma) and HeLa cells (cervical cancer). We knocked down AK4 expression in HeLa cells using short hairpin RNA and found that ATP production and their sensitivity to hypoxia and drugs increased. In subcutaneous grafting experiments using nude mice, HeLa cells with AK4 knockdown proliferated more slowly and exhibited a stronger response to cis-diamminedichloro-platinum(II) (CDDP). Metabolome analysis showed significant upregulation of fumarate and malate in AK4 knockdown cells, and significant downregulation of succinate, fumarate, and malate in AK4 overexpressing cells. This study showed that AK4 was involved in hypoxia tolerance, drug resistance, and mitochondrial control. These findings may lead to the development of efficient anticancer therapies by controlling AK4 expression.

P-0522

Association between sirtuins expression and hepatocellular carcinoma**Shingo Nakamoto^{1,2}, Tatsuo Kanda², Shuang Wu², Reina Sasaki², Yuki Haga², Masato Nakamura², Nan Nwe Win¹, Osamu Yokosuka², Hiroshi Shirasawa¹**¹Department of Molecular Virology, Graduate School of Medicine, Chiba University, Chiba, Japan; ²Department of Gastroenterology and Nephrology, Graduate School of Medicine, Chiba University, Chiba, Japan

Background: Several sirtuins (SIRT) have been reported to be associated with age-related disease including cancer. However, its role in hepatocellular carcinoma (HCC) is not fully understood. We analyzed the association between sirtuins expression and HCC.

Methods: For gene expression analysis, 44 human liver cDNAs (23 HCCs and 16 non-tumors, and 5 cirrhotic livers) in Tissuescan cDNA arrays (Origene, MD, USA) were used. SIRT1-7 expressions were detected using real-time PCR and normalized to beta-actin.

Immunohistochemistry (IHC) staining was performed against SIRT using 104 cases of liver tissue microarray (US Biomax, MD, USA). The array comprises 32 pair of HCC and non-tumor cirrhotic tissues, 24 cases of hepatitis and 16 normal cases. The intensity and quantity of antibody staining were compared. Chi-square test, *t* test, or Pearson correlation coefficient was used for statistical analysis. $p < 0.05$ was defined as significant.

Results: All SIRT1-7 mRNA expressions were detected in human liver cDNA samples. Among them, mitochondrial sirtuin SIRT5 expressions in HCC tissues were significantly down-regulated by 3.2 fold ($p < 0.0001$), compared to non-tumor and non-cirrhotic samples. SIRT5 expression levels was inversely associated with histologic grade; the expression in the well, moderate, and poorly differentiated tumors decreased by 1.73 ($P = 0.03$), 4.22 ($p < 0.0001$), 5.54 ($p < 0.0001$) fold, respectively, compared to non-tumor samples. In IHC analysis with SIRT5 antibody, more than 5 % of cells were stained positive in 10 % of HCC cases, while the same is true for 57 % of normal tissues ($p < 0.001$).

Conclusions: SIRT5 expression was altered in HCC and may be associated with hepatocarcinogenesis. Further investigations are needed.

P-0523

Functional genomics identified a novel EMT-MET regulator for human hepatoma invasion**Sen-Yung Hsieh^{1,3}, Ray-Ming Peng¹, Chih-Yung Chiou¹, Ming-Chin Yu²**¹Liver Research Unit, Chang Gung Memorial Hospital, Taoyuan, Taiwan; ²Department of General Surgery, Chang Gung Memorial Hospital, Taoyuan, Taiwan; ³Chang Gung University College of Medicine, Taoyuan, Taiwan

Background: Human hepatocellular carcinoma (HCC) is characterized by high frequency of local invasion and tumor recurrence. Epithelial-mesenchymal transition (EMT) is an initiating event driving tumor invasion and metastasis. However, the molecular mechanism of EMT in HCC remains largely unknown.

Methods: We have performed a human kinome/phosphatome RNAi screen to identify novel genes that are involved in invasion and metastasis of HCC cells using Hep3B, a low motile HCC cell line.

Results: We identified TIS1 (Tumor Invasion Suppressor 1) as a potential cell motility suppressor. TIS1 was frequently downregulated in HCC ($p = 0.001$). Downregulation of TIS1 is strongly associated with vascular invasion ($p = 0.013$), tumor recurrence ($p = 0.0052$, log-rank test), and poor prognosis ($p = 0.007$). Mechanistically, TIS1 is a novel suppressor of EMT. Silencing of TIS1 led to activation of the NOTCH signaling via induction of HIF1A and HIF2A. The activated Notch and HIF signaling pathways further induced EMT transcriptional reprogramming for tumor invasion and metastasis. By contrast, ectopic expression of TIS1 converted the EMT-resulting tumor cells to undergo the mesenchymal-epithelial transition (MET) for secondary tumor colonization and formation at the target sites. Suppression of the activated Notch or HIF signals inhibited tumor invasion and metastasis *in vitro* as well as *in vivo*.

Conclusion: TIS1 is a novel tumor invasion suppressor that regulates the transition between epithelial and mesenchymal traits of cancer cells for invasion and colonization during tumor metastasis. The TIS1-mediated EMT-MET signaling pathways may serve as therapeutic targets for prevention and treatment of HCC invasion and metastasis.

P-0524

Effect of raft-associated TLR7 activation on proliferation and migration of hepatocellular carcinoma

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Chronic liver disease is a predisposing factor for development of hepatocellular carcinoma (HCC). Toll-like receptors (TLRs) have an important role in innate immune responses and TLR signaling has been associated with various chronic liver diseases. Lipid rafts provide the necessary microenvironment in order for certain specialized signaling events to take place, such as the innate immune recognition. The purpose of this study was to determine the pattern of TLR7 expression in HCC, how recruiting TLR7 into lipid rafts responded to ligands and whether targeting TLR7 might have beneficial effects. The study group was comprised of 130 human liver tissues: 23 chronic hepatitis B (CHB), 18 liver cirrhosis (LC), 68 HCC and 21 normal livers. The expression of TLR7 was evaluated using immunohistochemistry, western blotting, and flow cytometry. Proliferation and migration of human HepG2 cells were studied following stimulation of TLR7 using the agonist gardiquimod and inhibition with a specific antagonist 20S-protopanaxadiol (aPPD). The activation of lipid raft-associated TLR7 signaling was measured using western blotting, double immunohistochemistry and immunoprecipitation in liver tissues and HepG2 cells. TLR7 expression was up-regulated in human HCC tissues and hepatoma cell line. Proliferation and migration of HepG2 cells in vitro increased significantly in response to stimulation of TLR7. TLR7 inhibition using aPPD significantly reduced HepG2 cell migration in vitro. The lipid raft protein caveolin-1 and flotillin-1 were involved with enhanced TLR7 signaling in HCC.

Conclusion: The data suggest that inhibiting TLR7 with antagonists, like aPPD, could potentially be used as a novel therapeutic approach for HCC.

P-0525

Human amniotic epithelial cell exosomes reduce proliferation of hepatocellular carcinoma cell lines

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Hepatocellular carcinoma (HCC) arises from cirrhotic liver driven by chronic inflammation, oxidative stress and high proliferative signals in the diseased liver. We have shown that human Amniotic Epithelial cell (hAEC) exosomes have immunomodulatory and anti-fibrotic properties in animal models. We hypothesize that they also have a direct effect on the carcinogenic transformation of cancer. We assessed the impact of hAEC exosomes on Huh-7 cell line (representing moderately differentiated HCC) and Sk-Hep1 cell line (representing poorly differentiated HCC). A Transwell culture protocol was used to coculture hAEC with both the HCC cell lines, allowing factors less than 40um diameter to pass through. Cell proliferation was assessed using Alamar Blue Staining over 4 days. Cell

motility was assessed via wound healing assay. Immunofluorescence staining for vimentin and EPCAM as markers of EMT was performed. The proliferation of Huh-7 was reduced in the hAEC arm compared with control (5842 vs 9042 units on Alamar Blue Stain). The proliferation of Sk-Hep1 was reduced in the hAEC arm compared with control (2264 vs 3113). The wound healing assay demonstrated reduced cell motility in the hAEC arm compared to control for Huh-7 (67.0 vs 82.1 % healing). Wound healing was also reduced in the hAEC arm vs control in SK-Hep1 (51.5 vs 70.1 %). Immunofluorescence staining for both vimentin and EPCAM were reduced in Huh-7 and Sk-Hep1 cells treated with hAEC. hAEC exosomes have the potential to retard proliferation, motility and reverse EMT. The ability to define and harness exosomes has tremendous potential for treatment of HCC.

P-0526

Increased expression of mitochondrial dynamics protein Drp1 in hepatocellular carcinoma

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Mitochondria play a critical role to maintain physiological functions of cells and dysfunction of mitochondria has been associated with tumorigenesis in recent years. In our previous studies, we found Mitofusin-2 (Mfn2), a mitochondrial fusion protein, expressed lower in hepatocellular carcinoma (HCC) tissues, which induced apoptosis in tumour cells. However, whether mitochondria dynamics involve in HCC remains speculative. In the present study, we evaluated the expression of the mitochondrial fission protein dynamin-related protein 1 (Drp1) in a series of HCC samples by quantitative PCR, and western blot. A marked upregulation of Drp1 was found in HCC tissues compared to adjacent normal tissues, while the mRNA level of Drp1 was reduced. Moreover, siRNA-mediated DRP1 knockdown and the pharmacological blockage of mitochondria division with mitochondrial division inhibitor-1 (Mdivi1), which was originally reported as an inhibitor of Drp1, had an inhibitory effect on cell proliferation, induced apoptosis and inhibited the migration of HCC cells in vitro. In conclusion, our findings may support the involvement of mitochondria dynamics in hepatocarcinogenesis and provide a novel way to understand the role of mitochondria in tumorigenesis.

P-0527

Nuclear Met regulates liver cancer metastasis via NF-κB/MMP2 pathway

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Met is a surface receptor tyrosine kinase which upon hepatocyte growth factor induction, triggers a diversity of downstream signaling cascades that modulates different cellular processes. Emerging evidence has shown the presence of nuclear Met (nMet) in some cancerous tissues and cell lines, postulating that nMet could have unexplored functions within nucleus. Although Met is well known of its oncogenic role in hepatocellular carcinoma (HCC), existence and

functions of nMet in HCC are yet to be reported. Our present study aims to examine the clinical association and functions of nMet in HCC. Immunohistochemistry of 103 human HCC paired samples showed that nMet was overexpressed in nearly 90 % of cases. We also found that nMet overexpression was progressively increased along HCC development, from non-tumorous liver tissue to early and advanced HCC. Nonetheless, nMet overexpression was significantly associated with venous invasion and poorer overall survival in HCC patients. As revealed by immunoblot and immunofluorescence, we found that nMet is about 48 kDa and comprises cytoplasmic domain of Met, as it can only be detected by an antibody against the carboxyl terminal of Met. To study the functions of nMet, we employed a lentiviral based inducible expression system to express the cytoplasmic region of Met. In vitro functional assay showed that nMet significantly promoted HCC cell proliferation and anchorage independent growth. It also significantly augmented HCC cell migration and invasiveness. Besides that, nMet also enhanced HCC tumor formation in animal model. Furthermore, we showed that nMet promoted tumor invasiveness and aggressiveness through NF- κ B/MMP2 pathway.

P-0528

PTP-PEST controls liver cancer cell migration through regulation of RhoA and Rac1

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Abstract: PTP-PEST is a cytoplasmic protein-tyrosine phosphatase (PTP) that has a role in cell motility, cytokinesis, focal adhesion turnover, and possibly the metastasis of cancer cells. Although the accumulating evidence supports a role of PTP-PEST in migration in T cells and fibroblasts, it is not clear how it contributes to control cancer cell migration. We show here that PTP-PEST gene silencing by short hairpin RNA is linked to the control of liver cancer cell (HepG2) adhesion and migration rather than to the regulation of cell proliferation. Cell motility is regulated by a balance between forward protrusion and tail retraction. These phenomena are controlled by a spatial asymmetry in signals at the front and the back of the cell. We show here that PTP-PEST is required for the coupling of protrusion and retraction during HepG2 cell migration. PTP-PEST shRNA HepG2 cells, which are blocked in migration, exhibit exaggerated protrusions at the leading edge and long, unretracted tails in the rear. This altered morphology is accompanied by changes in the activity of Rho GTPases, Rac1 and RhoA, which mediate protrusion and retraction, respectively. PTP-PEST shRNA HepG2 cells exhibit enhanced Rac1 activity and decreased RhoA activity. These findings point to a new mechanism of liver cancer progression leading to invasion and metastasis and suggest that the PTP-PEST signaling pathway could represent a new target for cancer therapy.

P-0529

Inhibition effect for the growth of hepatocellular cell line fibroblast growth factor (FGF) 5

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We investigated the quantitative profiles of FGFs and FGFRs in hepatocellular carcinomas (HCC) and hepatoblastomas compared with normal liver cells. Several FGFs and its functional receptors were highly expressed in hepatocellular carcinoma cell lines even though the FGFs are not checked from normal liver cDNAs. One of them is FGF5 and we focused it as it is known to have the mitogenic and cell survival activity in the cancer. We studied to check the inhibition effect for hepatocellular carcinogenesis by interfering FGF5 activity with its siRNAs in the HCC cell lines. We knocked down the level of FGF5 using siRNA technology and measured the extent of cell proliferation and apoptosis in HepG2 cells. We checked the Erk1/2 activity in the FGF5 silencing cells comparing to that of control cell. We also investigated the addictive effect of FGF5 silencing on the cytotoxicity in the combination treatment of anti-cancer drug, Cisplatin. FGF5 silencing in HepG2 cell lines caused profound decrease the cell viability and Erk activation upon the serum stimulation. Cisplatin treatment in the FGF5 silencing cells showed dramatic increase the cytotoxicity resulting in the cellular apoptosis. These results showed that hepatocellular carcinogenesis may due to specific expression of several FGFs and its counterpart receptors. We also propose impairment of FGF5 function in the HCC may critical for liver cancer therapy.

P-0530

ASPH disrupts mitochondrial DNA stability and mitochondrial function in hepatocellular carcinoma

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Background and Aims: Increased expression of aspartyl (asparagyl) beta-hydroxylase (ASPH) has previously been reported in hepatocellular carcinoma (HCC) and associated with tumor invasiveness. We identified an unexpected mitochondrial localization of ASPH which led us to investigate whether ASPH contributes to mitochondria dysfunction in HCC.

Methods: We identified the subcellular localization of ASPH through an analysis of fractionized mitochondrial proteins by immunoblotting and co-immunostaining with mitochondrial biomarkers. Mitochondrial DNA (mtDNA) integrity, characterized by the amount of D-loop mutation and copy numbers of D-loop and ND1 fragments, was analyzed in 71 HCC tissues and correlated with tumor ASPH expression level and clinicopathological features. The influence of ASPH to mtDNA integrity and mitochondrial function was testified in HCC cells overexpressed or knocked down with this gene. We then used mass spectrometry to identify proteins interacting with ASPH and investigate their impact on mtDNA integrity.

Results: We identified a mitochondrial localization of ASPH in HCC cells. Moreover, ASPH over-expression was correlated with decreased copy numbers of D-loop and ND-1 and enhanced D-loop mutation. Reduced mtDNA copy number was associated with aggressive pathological features of HCC. Enforced expression of ASPH in HCC cell lines also resulted in loss of mtDNA integrity and accompanied mitochondrial dysfunction. We further found that ASPH interacted with H2AX and over-expression ASPH diminished H2AX interaction with mitochondrial transcription factor A (mtTFA), resulting in reduced mtTFA binding to D-loop region.

Conclusion: ASPH over-expression disrupted the mtDNA integrity through H2AX-mTFA signal, thereby affecting mitochondrial functions and the disease progression.

P-0531

Association of STAT3 and STAT4 polymorphisms and hepatocellular carcinoma

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Background: Hepatitis B virus (HBV) infection is the leading cause of hepatocellular carcinoma (HCC) development. Recent studies demonstrated that single nucleotide polymorphisms (SNPs) rs2293152 in signal transducer and activator of transcription 3 (STAT3) and rs7574865 in signal transducer and activator of transcription 4 (STAT4) were associated with chronic hepatitis B (CHB)-related HCC in the Chinese population. We hypothesized that these polymorphisms might be related to HCC susceptibility in Thai population as well.

Methods: Study subjects were divided into 3 groups consisting of CHB-related HCC (n = 192), CHB without HCC (n = 200) and healthy controls (n = 190). The studied SNPs were genotyped using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP).

Results: The results showed that the distribution of different genotypes for both polymorphisms were in Hardy–Weinberg equilibrium ($P > 0.05$). Our data demonstrated positive association of rs7574865 with HCC risk when compared to healthy controls under additive model (GG versus TT: odds ratio (OR) = 2.07, 95 % confidence interval (CI) 1.06–4.03, $P = 0.033$). This correlation remained significant under allelic and recessive models (OR = 1.46, 95 % CI 1.09–1.96, $P = 0.012$ and OR = 1.71, 95 % CI 1.13–2.59, $P = 0.011$, respectively). However, no significant association between rs2293152 and HCC development were observed.

Conclusion: These data suggested that SNP rs7574865 in STAT4 might contribute to HCC progression in Thai population.

P-0532

Molecular mechanism of concanavalin A-induced autophagic cell death of human hepatoma cells

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Concanavalin A (ConA) is a lectin that can activate immune responses. Recently, ConA-induced autophagic cell death of hepatoma cells has been reported. However, the molecular mechanism of ConA-induced autophagic cell death is still unclear. Because macrophage migration inhibitory factor (MIF) can trigger autophagy in human hepatoma cells, the possible involvement of MIF in ConA-induced autophagic cell death was investigated. We demonstrated that LDH release is followed by an increment in MIF expression and secretion in the ConA-stimulated human hepatoma cell lines. In addition, ConA-induced autophagic cell death of hepatoma cells were blocked in the presence of a MIF inhibitor. Knockdown of endogenous MIF by shRNA confirmed that MIF is required for ConA-induced autophagic cell death of hepatoma cells. Furthermore, signal pathway studies demonstrated that ConA induces STAT3 phosphorylation to trigger MIF up-regulation, which in turn promotes BNIP3-dependent autophagy. By using a murine *in situ* hepatoma model, we further demonstrated that MIF contributes to anti-hepatoma activity of ConA. In summary, our findings uncover a novel role of MIF in lectin-mediated anti-hepatoma activities by regulating autophagy.

P-0533

ANGPTL6 inhibites hepatocarcinoma metastasis by promoting Wnt pathway through macrophages

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Background: The major purpose of ANGPTL6 gene therapy is inhibiting metastasis through regulation of cancer metabolism. We studied the metabolism related cytokine ANGPTL6 in this study. ANGPTL6 is closely related to hepatocarcinoma and regulates metabolism in lipid related diseases.

Methods: ANGPTL6-expressing metastatic hepatocarcinoma cell line Huh-7-ANGPTL6 was constructed. We detected changes in Wnt-pathway apoptosis-related factors and also investigated the effect of ANGPTL6 on hepatocarcinoma by injected Huh-7-ANGPTL6 and Huh-7-control fluorescence cells into tail vein in C57BL/6 mice. Metastasis was evaluated by image capture machine later. Immunohistochemistry was performed to analyze whether ANGPTL6 expression regulates the infiltration of macrophages in metastatic tissues.

Results: ANGPTL6 expression enhanced apoptosis through activation of Wnt pathway related apoptosis. Several factors related to Wnt pathway and caspase-3-mediated apoptotic pathway changed in metastasis tissues. We focus on FGF21, which was a metabolism regulator during several changed genes. We confirmed the relationship between ANGPTL6 expression and FGF21. Silencing FGF21 significantly decreased Wnt pathway related apoptosis. Metastasis of Huh-7-ANGPTL6 cells growth was inhibited by apoptosis induced by macrophages. Our findings suggest that the enhancement of apoptosis induced by ANGPTL6 and macrophages accumulation might inhibit hepatocarcinoma metastasis.

Conclusion: Our study reveals that ANGPTL6 regulates hepatocarcinoma metabolism and cell signaling. Therefore the role of ANGPTL6 in intracellular signaling pathway of hepatocarcinoma was step forward.

P-0534

Genetic polymorphisms in the Wnt pathway as prognostic predictors in HBV-associated HCC**Hyo Jung Cho, Soon Sun Kim, Choong Kyun Noh, Sung Won Cho, Jae Youn Cheong**

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Purpose: Wnt/beta-catenin signaling has a pivotal role in the pathogenesis of hepatocellular carcinoma (HCC). The present study aimed to determine whether genetic variation in the Wnt/beta-catenin signaling pathway is associated with the development and/or progression of HCC and the survival of patients with hepatitis B virus (HBV)-associated HCC.

Methods: We assessed seven single nucleotide polymorphisms (SNPs) of the AXIN1, AXIN2, CTNNB1, and WNT2 genes in 245 patients with HBV-associated HCC and 483 chronic HBV carriers without HCC. We analyzed the association of each SNP with HCC development or progression and overall survival.

Results: The CTNNB1 rs3864004 A allele was associated with a decreased risk of HCC development ($P = 0.049$). Haplotype analysis revealed a significantly higher frequency of CTNNB1 ht2 GA/GA in patients with HCC than in chronic HBV carriers without HCC ($P = 0.042$). The AXIN1 rs1805105 T to C SNP was associated with small tumor size and early tumor stage and the WNT2 rs39315 G allele was associated with early tumor stage in HCC. In Kaplan–Meier analysis, carriers of the AXIN1 rs214252 C allele showed longer survival than those with the TT genotype ($P = 0.020$). In multivariate Cox regression analysis, absence of CTNNB1 ht3 AA and advanced tumor stage were independent poor predictors of patient survival in patients with HCC.

Conclusion: These findings suggest that the genetic polymorphisms in Wnt/beta-catenin pathway genes might affect tumor development and survival in patients with HBV-associated HCC.

P-0535

Identification of novel liver cancer fusion genes using chromosome breakpoints screening**Chian-Feng Chen, Chin-Hui Lin**

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Gene fusion due to rearrangement or translocation of chromosomes is a powerful mutation mechanism during tumorigenesis. Several new high-resolution technologies have recently been developed to evaluate large numbers of small aberrations as candidate loci for fusion gene screening. In our previous whole-genome screening study using 500 k SNP arrays, we identified more than 700 homozygous deletions (HDs) and amplicons in 23 cancer cell lines. To explore novel fusion genes in hepatocellular carcinoma (HCC), we established stringent criteria for defining HD and amplicons breakpoints. Then we applying genomic PCR and sequencing analyses to predict a fusion gene, FNDC3B-PRKCI, was resulted by chromosome inter-rearrangement. Western blotting and 3'-RACE analyses revealed the chimeric transcript was an in-frame fusion between FNDC3B and PRKCI. Finally, cell migration and colony formation assays suggested that FNDC3B-PRKCI is a potential oncogene in HCC.

P-0536

Study of DLC1 regulates oxidative stress for tumor suppression**Frankie C. F. Ko¹, William C. S. Tai², Judy W. P. Yam¹**¹Department of Pathology, The University of Hong Kong, Pok Fu Lam, Hong Kong; ²School of Chinese Medicine, Baptist University of Hong Kong, Kowloon, Hong Kong

In our previous studies, we demonstrated that PKA/cAMP and PKB/Akt signaling pathways phosphorylate and regulate human DLC1 with opposite effect. PKB/Akt pathway attenuates DLC1 activity in a RhoGAP independent manner. In contrast, PKA/cAMP pathway activates DLC1 tumor suppressive functions through dimerization. In order to explore which downstream components involving these pathways, we performed MS/MS mass spectrometry and 2D-PAGE experiments using DLC1 immuno-precipitated proteins and total cell extracts respectively. These downstream candidates were screened and shortlisted for further bioinformatics analysis. These candidates are involved in glycolysis and TCA cycle of metabolic pathway to generate oxidative species and induce apoptosis. From our preliminary study, DLC1 induces acyl-CoA dehydrogenase (ACADM) and acyl-CoA Oxidase 1 (ACOX1) to generate oxidative stress for tumor suppressive functions. As DLC1 serves as an important tumor suppressor in hepatocellular carcinoma, we believe our study will substantially advance our understanding of the molecular basis of DLC1-dependent pathway in HCC development. More importantly, the study of the regulation of DLC1 downstream targets genes in metabolic pathway will have profound implications for therapeutic interventions for HCC (this study is supported by Small Project Funding/HKU 201309176089).

P-0537

In vivo study on the roles of HBx and HCP in pathogenesis by conditional mouse models**Hsiang-Hsuan Fan¹, I-Shing Yu^{2,3}, I-Chang Su⁴, Chang-Ching Lin⁴, Shu-Waha Lin^{2,5}, You-Tzung Chen¹**¹Graduate Institute of Medical Genomics and Proteomics, National Taiwan University College of Medicine, Taipei, Taiwan; ²Transgenic Mouse Models Core, National Core Facility Program for Biotechnology, Ministry of Science and Technology, Taipei, Taiwan; ³Laboratory Animal Center, National Taiwan University College of Medicine, Taipei, Taiwan; ⁴Graduate Institute of Clinical Medicine, National Taiwan University College of Medicine, Taipei, Taiwan; ⁵Department of Clinical Laboratory Science and Medical Biotechnology, National Taiwan University College of Medicine, Taipei, Taiwan

Liver cancer is the second leading cause of cancer-related death in Taiwan. Chronic HBV and HCV infections are the major risk factors for hepatocarcinogenesis. Co-infection with HBV and HCV is found highly associated with more severe liver disease and higher risk to develop HCC. Among the hepatitis virus-encoded proteins, HBV × protein (HBx) and HCV core protein (HCP) are indicated to be important in hepatocarcinogenesis in transgenic studies. There are several HBx- and HCP-interacting proteins identified by in vitro studies, but lack in vivo evidence to verify. Hepatic progenitor cells (also called oval cells in rodents) activation is shown in the majority

of chronic liver disease and is associated with severity of liver disease. It is still not clear that if hepatic progenitor cells can be the liver cancer initiating cells. Here, we generated mice conditionally over-express HBx and/or HCP in either liver or oval cells to facilitate research on hepatitis virus-induced hepatocarcinogenesis. To study the synergistic contribution to tumorigenesis in dual infection of hepatitis B and C viruses, a transgenic mouse expressing both HBx and HCP in liver was generated. To provide direct, in vivo, evidence that oval cells can serve as the cellular origin of HCC, we used a mouse model overexpressing virus-encoded protein specifically in oval cells. Our mouse models may help to uncover the mechanism of hepatitis virus-related hepatocarcinogenesis and may serve as a new platform for preclinical therapeutic treatment evaluation in the future.

P-0538

Screening of c-Met-positive hepatocellular carcinoma for c-Met-based target therapy

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Hepatocellular carcinoma (HCC) is one of the most common causes of death from cancer worldwide. Surgery of small HCC may result in marked increase in 5-year survival rate from 20 to 50 %. However, the poor prognosis and recurrence of HCC are very high, due to intrahepatic and extrahepatic metastasis. Hepatocyte growth factor (HGF) is well known to be a metastatic factors secreted in tumor microenvironment. Mounting studies highlights the essential role of HGF/c-MET axis in driving the tumor progression of HCC. Therefore, c-Met represents a potential therapeutic target for hepatocellular carcinoma. However, one of the major concerns is that HGF/c-Met signaling may not be activated in all HCC tissue. For those HCC with negative c-Met signaling, c-Met targeting approach will not be adequate. On the other hand, side effects of c-Met targeting are often encountered. Therefore, firstly, screening of HCC with active c-Met signaling can be performed to enroll suitable patients for treatment. In our study, we have found 45 % of our patients with HCC expressed c-Met. Using a suitable c-Met inhibitor to these HCC patients is more promising than non-specific use of it. Secondly, effective c-Met inhibitors that warrant safety can be carefully selected for suppressing tumor progression of HCC with active c-Met signaling. We will demonstrate that this is an effectively personalized approach for the treatment of HCC.

P-0539

DPP-4 inhibitor, anagliptin suppresses the progression of Hepatocellular carcinoma

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Background and Aim: CD 26 is a multifunctional transmembrane glycoprotein and functions as dipeptidyl peptidase 4 (DPP-4). CD26 is expressed in various cancers, but the relationship between hepatocellular carcinoma (HCC) progression and CD26 expression

remains unknown. We investigated the potential role of CD26 as a molecular target for HCC treatment.

Methods: CD26 expression was examined in 41 surgically resected liver specimens from patients with HCC. In vitro the effect of DPP-4 inhibitor, anagliptin, on cancer cell proliferation and cell cycle was investigated using Huh7/HepG2 cells that expresses CD26. In vivo nude mice (BALBc-nu/nu) were subcutaneously injected Huh7 cells and then fed control diet, low-dose anagliptin containing diet or high-dose anagliptin containing diet for 21 days.

Results: CD26 expression was correlated with cell proliferation, angiogenesis and cell differentiation in HCC specimens. Although anagliptin did not affect cell proliferation or cell cycle in vitro, it significantly suppressed the growth of xenograft tumors in dose dependent manner in vivo, suggesting that anagliptin potentially have antitumor activity against HCC. Anagliptin also induced NK cells infiltrations more vigorously by activating the chemotaxis of NK cells probably through the reduction of CXCL10 antagonist, because it has been shown that CXCL10 is truncated at its N-terminus through DPP-4 activity and that a N-terminal truncated CXCL10 acts as chemokine antagonist.

Conclusions: Our results showed that CD26 was related to tumor progression in HCC patients and that DPP-4 inhibitor, anagliptin potentially suppressed HCC progression through affecting microenvironment of HCC.

P-0540

Chronic apoptotic stimuli in hepatocytes induces oxidative stress leading to development of HCC

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Background/aim: Hepatocellular carcinoma (HCC) is frequently developed in livers with chronic hepatitis, in which hepatocytes suffer chronic apoptotic stimuli. However, the underlying mechanisms between apoptotic stimuli and carcinogenesis are not fully clarified.

Methods/results: Apoptotic stimulation by a Bad mimetic induced caspase activation and generates reactive oxygen species (ROS) in cultured hepatocytes. Mitochondria isolated from murine liver, upon administration of tBid, released not only cytochrome c but also ROS into the supernatant. Hepatocyte-specific Mcl-1 or Bcl-xL knockout mice, which suffer continuous apoptotic stimuli in their hepatocytes, increased the number of 8-OHdG-positive hepatocytes in their livers and developed HCCs over a year. Further genetic ablation of a pro-apoptotic protein, Bid, Bak or Bax, ameliorated apoptotic stimuli in the hepatocytes of Mcl-1 knockout mice. It decreased both the number of 8-OHdG-positive hepatocytes and the incidence rates of their liver tumors in Mcl-1 knockout mice. Whole exome sequencing could not find any specific mutated driver genes in HCCs of Mcl-1 knockout mice. However, it revealed that GC-TA transversion, which is associated with oxidative injury, was frequently observed in them, in consistent with human HCCs. The administration of antioxidant N-acetylcysteine into Mcl-1 knockout mice did not ameliorate their hepatocyte apoptosis but significantly reduced the incidence rates of their liver tumors.

Conclusion: Hepatocytes chronically suffering apoptotic stimuli accumulate oxidative stress. Some of them are suggested to developed malignant transformation by oxidative DNA damage leading to liver tumorigenesis. Antioxidant was indicated to be useful for suppressing liver carcinogenesis in patients with chronic liver disease.

P-0541

Inactivating mechanism of ATBF1 gene in hepatocellular carcinomas**Neung Hwa Park, Chang Jae Kim, Jung Woo Shin, Seok Won Jung, Bo Ryung Park**

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Background and aims: Alpha-fetoprotein (AFP) is frequently detected in hepatocellular carcinomas (HCCs) and AT motif binding factor 1 (ATBF1) down-regulates AFP gene expression in hepatic cells. The ATBF1 gene also inhibits cell growth and differentiation and altered gene expression is associated with malignant transformation.

Methods: To investigate the potential role of the ATBF1 gene in HCCs, we analyzed somatic mutations, allelic loss and hypermethylation of the ATBF1 gene in 45 sporadic HCCs. The level of ATBF1 mRNA expression was analyzed using quantitative real-time RT-PCR.

Results: Genetic studies of the ATBF1 gene revealed absence of a somatic mutation in the hotspot region and 5 (16.7 %) of 30 informative cases showed allelic loss at the ATBF1 locus. Hypermethylation in the intron 1 region of the ATBF1 gene was detected in only one case. Interestingly, ATBF1 mRNA expression in HCCs was significantly reduced in 33 (73.3 %) samples compared to the corresponding surrounding liver tissues.

Conclusion: These results suggest that the ATBF1 gene may contribute to the development of HCCs via transcriptional down-regulation of mRNA expression, but not by genetic or epigenetic alterations.

P-0542

Clinical characteristics of Glycine and Taurine exchange in patients with liver cancer**Alexandre Tavartkiladze^{1,2}, Gaiane Simonia^{1,2}**¹Department of Clinical Oncology of Tbilisi State Medical University, Tbilisi, Georgia; ²Georgian Cancer and Internal Medicine Research Center, Atlanta, Georgia

Each of 20 known amino acids plays particular biochemical role. They are one of the general indicators for evaluation of metabolic exchange. Our clinical observations have shown the obvious deficiency of Glycine and Taurine among the patients with Primary Liver Cancer (HCC). In our point of view, the deficiency of Taurine and Glycine should be explained by the fact, that they are essential substances which in the Hepatocytes, the Liver cells, form detoxifying complexes with bile acids, which contribute to further neutralization and elimination from the body. III and IV stage, 32 Patients with HCC under observation, underwent full screening of amino acids in the blood plasma. The control group consisted of 45 healthy patients. The results of the study have revealed that 79 % of patients have Taurine deficiency (30.2 ± 0.15) if compared with the control group (62.1 ± 0.45) and by 47.2 % Glycine deficiency (56.5 ± 0.15) if compared with control group (228.7 ± 0.2). As the result of the study, we can conclude that the toxins, existing in the body of Oncological patients, belong to the group of substances, which can be detoxified in the liver by Glycine and Taurine conjugation. Therefore, chronic oncological intoxication in the body causes active expenditure of Glycine and Taurine and their elimination with the urine. This

is proved by their increased values in the urine. It is not excluded for oncologic patients to have certain syndromes, caused by the deficiency of amino acids, which is evidenced by Glycine and Taurine, delivered by us (per os).

P-0543

Protein kinase R modulates DNA methylation in hepatocellular carcinoma with HCV infection**Takao Watanabe, Yoshio Tokumoto, Masashi Hirooka, Osamu Yoshida, Yohei Koizumi, Yusuke Imai, Yoshiko Nakamura, Masanori Abe, Yoichi Hiasa**

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PKR is overexpressed in hepatocellular carcinoma (HCC) with hepatitis C virus (HCV) infection. Recently, epigenetic alterations have been believed to constitute the progression of cancers. We aimed to identify whether the upregulated PKR in HCC with HCV infection could play an epigenetic role in the pathogenesis. We used the JFH-1 HCV-replicating cell line generated by Huh 7.5.1 cell transfection. With this cells, we modulated the expression of PKR using PKR-specific siRNA and PKR inhibitors. First, we examined whether PKR altered the expression of DNA methyltransferase (DNMT1, DNMT3a, and DNMT3b). We then evaluated methylation frequency and methylated genes by DNMTs using Human Methylation450 BeadChip™. Moreover, after modulating PKR expression, we analyzed the amount of mRNA of candidate genes with or without 5-aza-deoxycytidine (5-aza-dC) treatment. Expressions of DNMT1, DNMT3a, and DNMT3b mRNA were upregulated in JFH-1 with PKR siRNA ($p < 0.01$ each vs control siRNA). Comprehensive BeadChip analysis showed the total frequency of DNA methylation was upregulated in PKR-downregulated JFH-1 cells. Among the altered genes, suppressor of cytokine-6 (SOCS6), phosphatidyl 3,4,5-triphosphate Rac exchanger 1 (P-Rex1), and Ras-association domain family 1A (RASSF1A) genes showed high log ratio changes in methylation frequencies with downregulation of PKR. Amounts of mRNA for those genes were also downregulated by PKR inhibitor in real-time RT-PCR assays. After treatment with 5-aza-dC, alterations by PKR inhibitor were most markedly diminished for P-Rex1 mRNA. In conclusion, PKR could be associated with DNA methylation in HCC with HCV, and would contribute to the pathogenesis of HCC with HCV infection.

P-0544

An ssDNA Aptamer Selectively Delivers Cytotoxic Drug to Hepatocellular carcinoma Cells**Ge Yu^{1,2}, Huan Li^{2,4}, Yue Qi¹, Ruihong Wu^{1,3}, Hongqin Xu^{1,3}, Xiumei Chi^{1,3}, Xiaomei Wang^{1,3}, Xiuzhu Gao^{1,3}, Qinglong Jin¹, Yanhang Gao¹, Yu Pan¹, Janguo Wen², Junqi Niu¹, Youli Zu²**

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Hepatocellular carcinoma (HCC) is the fifth most common malignant tumor and the third most common cause of cancer-related mortality worldwide, with 50 % of cases occurring in China. Currently, molecular-targeted agents such as sorafenib could only extend the overall survival by 2–3 months. Plus, side effect such as diarrhea and weight loss were more frequent in the sorafenib treatment group. Aptamer-based tumor targeted drug delivery system is a promising approach that may increase the efficacy of chemotherapy and reduce the related toxicity. In this study, we developed a new HepG2-specific aptamer (HCA#3) by using systematic evolution of ligands by exponential enrichment technology (SELEX) and exploited its role as a targeting ligand for delivering doxorubicin (DOX) to HepG2 cells in vitro. The selected 76-nucleotide aptamer preferentially bound to HepG2 HCC cells but not other control cells. For drug delivery, aptamer HCA#3 was synthesized with CG-cargo to carry a high payload of DOX through non-covalent intercalation. Each molecule of the formed aptamer-doxorubicin conjugate (ApDC) was able to completely incorporate four DOX payloads (mol/mol). Biostability analysis showed that ApDC were stable at the physiological temperature and in human serum. Functional analysis showed that ApDC specifically targeted and released DOX within HepG2 cells, but not in control cells. ApDC treatment specifically induced apoptosis of HepG2 cells but had minimal effect on control cells. Taken together, our study demonstrates that HCA#3 ApDC is a new targeted therapeutic approach for specific delivery and intracellular release of a high drug payload in HCC cells.

P-0545

Effects of lin28B on human colorectal carcinoma and HBV-associated hepatocellular carcinoma

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Lin28 homolog B (lin28B), microRNA (miRNA) binding protein, is a marker of cancer stem cell. Lin28B regulates let-7c by binding to the terminal loop of let-7c precursors and blocks processing into mature miRNA, resulting in derepression of let-7c targets. In this study, we examined the role of lin28B in carcinogenesis of human colorectal carcinoma (CRC) and HBV-associated hepatocellular carcinoma (B-HCC). CRC, CRC adjacent tissues, HBV-associated liver cirrhosis (B-LC), B-HCC tissues or serum samples were collected. The expressions of lin28B and let-7 family miRNAs were investigated by real-time RT PCR or Microarray. We observed that lin28B mRNA levels were up-regulated in human CRC and C-HCC tissues, and we analyzed the expression of let-7 family miRNAs, found that the expression levels let-7a, -7c, -7d and -7f were higher than let-7b, -7e, -7g and -7i in CRC adjacent tissues, and the expression of let-7a, -7c, -7d and -7f were down-regulated in CRC tissues compared with in adjacent tissues. Especially, the expression of let-7c was significantly reduced (1.00 ± 0.064 vs. 0.717 ± 0.106 , $p < 0.05$). We also examined the expression of lin28B and let-7c in B-LC and B-HCC tissues or serum, found that the lin28B expression was increased in B-HCC tissues, and let-7c was down-regulated in B-HCC by real-time RT-PCR. However, the cell proliferation and c-Myc expression, a let-7c target gene, were enhanced in CRC or B-HCC tissues and in let-7c knockdown cells. Our results suggest that lin28B might play an

important role in carcinogenesis of human CRC and B-HCC, through mediating let-7c and c-Myc expression.

P-0546

LncRNA INTS6P1, a prognostic factor in HCC, induces apoptosis via intrinsic mitochondrial pathway

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Aims: We aimed to determine whether the lncRNA INTS6P1 could be as a tumor suppressor and its mechanism in hepatocellular carcinoma.

Methods: Firstly, the expression level of INTS6P1 mRNA were measured in a cohort of 60 HCC tissues and adjacent normal liver tissues by using qRT-PCR; Secondly, the functional studies of INTS6P1, include growth curves, migration assays and cell death, were detected in HCC cell lines. Finally, the mechanism experiment was investigated for tumor suppressive roles of INTS6P1.

Results: we found that INTS6P1 was down-regulated in HCC (71.4 %), and its expression was significantly correlated with pathology grade and tumor recurrence. Moreover, Kaplan–Meier analysis revealed that patients with the lower INTS6P1 expression had a shorter overall and disease-free survival than patients with high expression. Overexpression of INTS6P1 in HCC cells leads to the apoptotic features of cell nucleus, DNA fragmentation, and the increase of cell apoptotic rate, showing that INTS6P1 can induce apoptosis in HCC cells. By detecting the change of mitochondria distribution, mitochondrial membrane potential (MMP), and the cytochrome c release, we found that overexpression of INTS6P1 concentrated distribution of mitochondria became diffused and disordered, MMP and cytochrome c release reduced. After using microarray, bioinformatics, and qRT-PCR analysis, it could promote cytochrome c release, activation of caspase-3,9 and cell apoptosis in overexpression of INTS6P1 HCC cells.

Conclusions: Down-regulated INTS6P1 expression was associated with poorer prognosis in HCC patients and induced apoptosis by activating the intrinsic mitochondrial pathway. INTS6P1 maybe as a potential target and new therapy for promoting tumor cells' apoptosis.

P-0547

lncRNA CPS1-IT1 suppresses hepatocellular carcinoma metastasis by regulating HIF-1 α activity

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Background: Recently, increasing numbers of long noncoding RNAs (lncRNAs) have been found to be aberrantly expressed in various human cancers, with the potential for both oncogenic and tumor suppressive roles. However, the role of lncRNAs in hepatocellular carcinoma (HCC) progression is still largely unknown.

Methods: In this study, we performed a comprehensive microarray analysis of lncRNA expression in human HCC specimens. After validation in 119 human HCC tissues, we identified a novel tumor suppressor lncRNA named CPS1 intronic transcript 1 (CPS1-IT1). The correlations between CPS1-IT1 levels, clinical parameters, and survival outcomes were analyzed to elucidate the clinical significance of CPS1-IT1 in HCC. In vitro and in vivo functional assays were also performed to dissect the potential underlying mechanisms.

Results: It was found that the expression of CPS1-IT1 was significantly downregulated in 73 % of HCC tissues, and patients with lower expression of CPS1-IT1 had poor survival outcome. Furthermore, in vitro functional assays indicated that CPS1-IT1 significantly reduced cell proliferation, migration and invasion capacity via reduced binding and activation of Hsp90 with HIF-1 α , thereby suppressing epithelial-mesenchymal transition. An in vivo animal model also demonstrated the tumor suppressor role of CPS1-IT1 via a reduction in tumor growth and metastasis.

Conclusions: lncRNA-CPS1-IT1 acts as a tumor suppressor in HCC via the reduction of HIF-1 α activation and suppression of epithelial-mesenchymal transition. The findings of this study establish a function for CPS1-IT1 in HCC progression and suggest its candidacy as a new prognostic biomarker and potential target for HCC therapy.

P-0548

Differential gene expression of miRNA in plasma MV of patients with hepatocellular carcinoma

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Background: Hepatocellular carcinoma (HCC) is a hypervascular neoplasm with high levels of apoptosis and necrosis. To date there has been a paucity of early diagnostic plasma biomarkers for HCC. Microvesicles (MVs) are sub-micron vesicles secreted from cell plasma membrane in both physiological and pathological states. We investigated the utility of MV as a diagnostic and prognostic biomarker for HCC.

Methods: MVs were isolated from human plasma using differential centrifugation protocols, and enumerated using NanoSight. MV total RNA was extracted from patients with cirrhosis (n = 8), HCC (n = 8), and viral hepatitis (n = 5). miRNA expression were measured with the nCounter system (NanoString), differentially expressed miRNA target genes and signalling pathways were predicted by bioinformatics analysis.

Results: MV concentration in plasma of HCC patients was significantly increased by 3.4 fold compared with normal human plasma MV (p < 0.0001). Expression of 798 miRNA within MVs were measured using the nCounter system, and a significant differentially expressed gene profile was found in plasma MV of HCC patients compared with normal plasma MVs, normal whole plasma, and normal human liver. Pathway analysis demonstrated HCC MVs contain miRNAs targeting genes involved in the upregulation of p53 mutation (p < 0.0001) and PI3 K/akt/mTOR signalling (p < 0.0001).

Conclusion: MV secretion is increased in patients with hepatocellular carcinoma. There are unique miRNA profiles contained within circulating MV arising in HCC. Major carcinogenesis pathways, including p53 and PI3 K/akt/mTOR, are implicated in differentially expressed miRNA gene signature of MVs in HCC. These may have prognostic and therapeutic implications for genomics-guided management of HCC.

P-0549

Involvement of autophagy in resistance to sorafenib induced by hypoxia in hepatocellular carcinoma

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Backgrounds: Sorafenib inhibits angiogenesis which could lead the tumor into severe hypoxia. Hypoxia is related to resistance to many types of anticancer drugs. We investigated the involvement of autophagy in the hypoxia-induced resistance to sorafenib in hepatocellular carcinoma (HCC).

Methods: HCC cell lines, PLC/PRF/5 (PLC5), Li7, HepG2, and Huh7 were cultured in 95 % air and 5 % CO₂ (normoxia) or in 94 % N₂ with 1 % O₂ and 5 % CO₂ (hypoxia). Anchorage dependent proliferation and induction of apoptosis were assessed. Involvement of autophagy was assessed by detecting LC3 and p62 and by treating cells with chloroquine (Cq), an inhibitor of autophagy.

Results: Sorafenib inhibited proliferation of cells in a concentration-dependent manner and this effect was reduced under hypoxia with an IC₅₀ (normoxia/hypoxia, μ M) of 5.9/9.4 in PLC5, 7.4/11.4 in Li7, 12.1/14.5 in Huh7, and 6.9/11.7 in HepG2. Incubation with sorafenib (5 and 10 μ M) for 24 h induced apoptosis by 14.6 and 23.8 % in PLC5 in normoxia whereas 10.5 and 13.1 % in hypoxia. An increase of caspase-3/7 activity induced by sorafenib (5 and 10 μ M) was reduced by 77.2 and 55.3 % under hypoxia. Co-treatment with Cq (50 μ M) recovered the cytotoxicity of sorafenib (10 μ M, 72 h) by 70.9 % in PLC5 and 67.3 % in Li7.

Conclusion: Autophagy is involved in hypoxia-induced resistance to sorafenib. Inhibition of autophagy could be an attractive approach for potentiating the antitumor effect of sorafenib in HCC.

P-0550

Thyroid hormone-activated DAPK2 suppresses hepatocarcinogenesis through autophagy

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Recent studies have demonstrated a critical association between disruption of cellular thyroid hormone (TH) signaling and the incidence of hepatocellular carcinoma (HCC), but the underlying mechanisms remain largely elusive. Here, we showed that disruption of TH production results in a marked increase in progression of diethylnitrosamine (DEN)-induced HCC in a murine model, and conversely, TH administration suppresses the carcinogenic process via activation of autophagy. Treatment with chloroquine (CQ), an inhibitor of autophagy, suppressed the protective effects of TH against DEN-induced hepatic damage and development of HCC. The involvement of autophagy in TH-mediated protection was further supported by data showing transcriptional activation of death-associated protein kinase 2 (DAPK2, a serine/threonine protein kinase) in TH-treated hepatocytes. Ectopic expression of DAPK2 further attenuated DEN-induced hepatotoxicity and DNA damage though enhanced autophagy. The pathological significance of the TH-mediated hepatoprotective effect by DAPK2 was confirmed by the concomitant decrease in expression of TR and DAPK2 in matched HCC tumor tissues. Taken together, these findings indicate that TH

promotes hepatocyte autophagy via induction of DAPK2, in turn, protecting against DEN-induced hepatotoxicity or carcinogenesis.

P-0551

BTLA up-regulation impaired CD8+ T-cell function and accelerated disease progression of HCC patients

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Background: B- and T-lymphocyte attenuator (BTLA) negatively regulates immune responses; however, it remains unknown the expression profile and functional role of BTLA in patients with hepatocellular carcinoma (HCC).

Methods: One-hundred and fifty HCC patients, 33 liver cirrhosis (LC) patients, and 45 healthy individuals were enrolled in the study. Flow cytometry performed to analyze the properties of circulating, tumor- and non-tumor-infiltrating BTLA+ cytotoxic CD8+ T cells in these HCC patients with various stages of disease progression. The Mann–Whitney U test, Kruskal–Wallis H test and Wilcoxon signed ranks test were used to compare groups.

Results: We found that BTLA expression was progressively up-regulated on cytotoxic CD8+ T cells in peripheral blood and correlated with disease progression in HCC patients. And tumor-infiltrating cytotoxic CD8+ T cells had higher BTLA expression compared with non-tumor regions. Further analysis revealed that BTLA expression was negatively correlated with granzyme and perforin expression in CD8+ T cells both in peripheral blood and liver. And BTLA+CD8+ T-cell proliferation and degranulation were significantly decreased compared with BTLA-CD8+ T-cell. Importantly, increased BTLA expression on cytotoxic CD8+ T cells were associated with high recurrence rates after HCC resection.

Conclusions: These novel findings suggest that BTLA-mediated inhibitory function may play an important role in disease progression of HCC, and represent both a potential prognostic marker and a therapeutic target for the treatment of HCC.

P-0552

NANOG modulates chemoresistance of liver cancer stem cells by activating stat3/ABCB1 pathway

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Chemoresistance is a major reason for the failure of liver cancer chemotherapy in clinic. Cancer stem cells (CSCs), a subset of cancer cells that fuel tumor initiation, are highly resistant to current chemotherapies. NANOG is one of core transcription factors for modulating the maintenance of pluripotency and self-renewal in embryonic stem cells and CSCs. However, the relationship between NANOG and chemoresistance in liver CSCs remains unclear. The aim of this study was to investigate whether NANOG have a potential role in modulating chemoresistance of liver CSCs. In this study, liver cancer stem-like cells were purified from Hep3B human hepatoma

cells by side population (SP) cell sorting method. We found that NANOG, as well as phosphorylated stat3 and ABCB1 (MDR1, P-gp), were highly expressed in SP cells. Knockdown of NANOG led to enhanced chemosensitivity to doxorubicin and reduced expression of phosphorylated stat3 and ABCB1 in SP cells. In addition, treatment by stat3 pathway inhibitor also increased chemosensitivity to doxorubicin and decreased expression of phosphorylated stat3 and ABCB1 in SP cells. Furthermore, our ChIP experiment showed that phosphorylated stat3 protein can bind to the promoter of ABCB1 gene, indicating stat3 may regulate ABCB1 expression directly. Together, our findings suggest that NANOG may modulate chemoresistance of liver CSCs to doxorubicin by activating stat3/ABCB1 pathway, thus NANOG might represent a novel potential therapeutic target for liver cancer treatment.

P-0553

Saturated fatty acid induces cancer stem cell-like properties in human hepatoma cells

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Hepatic steatosis has been reported to be a risk factor for the development of liver cancer. The underlying mechanism of carcinogenesis remains to be elucidated. It has been postulated that cancer stem cells (CSCs) within tumor tissues are a subset of cells with stem cell properties of self-renewal and undifferentiation. The purpose of this study was to investigate the effects of a saturated fatty acid, palmitate (PA), on CSC-like properties of human hepatoma HepG2 cells. We investigated the effects of PA on HepG2 cells and primary rat hepatocytes (PRH) by exposing them to PA to induce lipid accumulation. Significant fat accumulation was observed by Oil Red O staining in cells exposed to PA, and it was accompanied by significant increase in NFκB (p65) nuclear translocation in HepG2 cells. Notably, PA significantly enhanced the sphere forming ability of HepG2 cells, but not PRH. Furthermore, PA significantly increased stemness gene expressions of Sox2 and Oct4, and production of sonic hedgehog (Shh). Moreover, NFκB inhibitors, *N*-Acetyl-L-cysteine and pyrrolidine dithiocarbamate, and a NOX inhibitor, diphenyleneiodonium, significantly attenuated PA-induced sphere forming ability of HepG2 cells. Our results suggest that lipid accumulation may not only induce pro-inflammatory responses in hepatocytes but may also activate CSC-like properties of hepatoma cells through NFκB activation.

P-0554

Sorafenib targets CD90+ cancer stem cells and controls distant metastasis

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Background: Cancer stem cells (CSCs) are a pivotal target for eradicating hepatocellular carcinoma (HCC). We reported that the CSC markers EpCAM and CD90 are expressed independently in primary HCCs and cell lines, and EpCAM+ cells share features with tumorigenic epithelial stem cells, whereas CD90+ cells share features of metastatic vascular endothelial cells. We examined the influence of sorafenib on EpCAM+ and CD90+CSCs.

Methods: Gene/protein expression was analyzed by microarray, qRT-PCR, western blotting, and FACS analysis. CD90+ and EpCAM+ CSCs were separated by MACS. Cell proliferation and motility were assessed by MTS assay and time-lapse imaging, respectively. Tumorigenicity and lung metastasis were evaluated in a subcutaneous xenotransplant model by injecting NOD/SCID mice with tumorigenic EpCAM+ cells and metastatic CD90+ cells.

Results: CD90+ cells showed higher c-Kit gene/protein expression with enhanced cell motility compared with EpCAM+ cells. Sorafenib treatment reduced the number of CD90+ cells with reduced c-Kit phosphorylation. Conversely, sorafenib enriched the population of EpCAM+ cells. When EpCAM+ and CD90+ cells were subcutaneously co-injected *in vivo*, EpCAM+ cells acquired the lung metastasis capacity *de novo* by the activation of TGF- β pathway mediated by CD90+ cells. Although subcutaneous tumor growth was slightly suppressed by sorafenib in this model, pulmonary metastasis was completely suppressed.

Conclusions: Sorafenib suppressed mesenchymal CD90+CSCs to inhibit metastasis through targeting c-Kit signaling, but had limited effects on epithelial tumorigenic EpCAM+CSCs. Differences in tumorigenic/metastatic capacity and sensitivity against sorafenib might be related to the presence of distinct EpCAM+/CD90+ CSCs in HCC.

P-0555

TGF- β 1 enhances EMT in CD44 HCC cell lines, leading to cancer progression and metastasis

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Background: The character of metastatic cells is strongly correlated to epithelial-mesenchymal transition (EMT) and cell adhesion molecules such as cadherin and CD44. CD44 is a receptor for hyaluronic acid, plays a role in invasion and metastasis. Moreover, TGF- β 1 is a multifunctional cytokine that induces the EMT and metastasis in HCC progression. This article investigates interplay between TGF- β 1 and CD44 during EMT in HCC cell lines.

Methods: We determined expression of CD44 through FACS. Also, we determined the expression of TGF- β 1 in SNU-354 and SNU-368. In TGF- β 1 treat cells and CD44 sorted cells, expression of EMT-related proteins detected by western blotting and cells plated allowed for sphere formation, migration assay.

Results: At the FACS analysis, the CD44 was expressed in high purity in SNU-354 (69.37 ± 4.96 %) and SNU-368 (82.09 ± 3.15 %) cell lines. TGF- β 1 was only expressed in SNU-368 but not in SNU-354. SNU-368 CD44+ cells show EMT, but were not induced in SNU-354 CD44+. Therefore, SNU-354 cells treated with TGF- β 1 and SNU-368 cells treated with TGF- β 1 inhibitor. The TGF- β 1-treated SNU-354 cells and SNU-368 CD44+ cells accompanied by loss of epithelial marker and gain of mesenchymal marker. Also, formed spheres and show enhances cell motility. Whereas, The TGF- β 1

knockdown in SNU-368 CD44+ cells inhibited sphere formation and cell motility.

Conclusions: TGF- β 1 increases the expression of EMT-related proteins with CD44 expression in SNU-354 cells. TGF- β 1 inhibitor induced loss of CD44 in SNU-368. Therefore, TGF- β 1 promotes the EMT of HCC cell lines with CD44 expression. Thus, co-expressed of TGF- β 1 and CD44 required for tumor metastasis.

P-0556

An EpCAM/NCAM expressing HCC in which progenitor marker positive cells are important for metastasis

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Introduction: Hepatic progenitor cell (HPC) marker-positive hepatocellular carcinomas (HCCs) have recently been extensively analyzed and their prognosis has been reported as poor compared to HPC marker-negative HCCs. However, previous studies have analyzed the existence of HPC marker-positive cancer cells only in primary lesions, as well as the recurrence rate and prognosis of such tumors. Here, we first report the behavior of HPC marker-positive cancer cells during vascular invasion and metastasis of a HCC. A HCC including EpCAM and/or NCAM expressing cancer cells has not been previously reported.

Methods: We concurrently analyzed EpCAM and/or NCAM expressing cancer cells in the primary, vascular invasion, and metastatic lesions of a HCC.

Case and Results: EpCAM and/or NCAM-positive cancer cells invaded into the vessels and formed heterogeneous populations of these HPC marker-positive cancer cells with HPC marker-negative cancer cells. The frequency of HPC marker-positive cancer colonies and cells in vessels was higher than that in the primary HCC. In the metastatic lesions, EpCAM-positive cancer cells were more frequently detected than NCAM-positive cancer cells indicating that EpCAM is more important than NCAM for cancer cell settlement in the metastatic lesions. Furthermore, bigger metastatic tumors tended to include HPC marker-positive cancer cells, suggesting that HPC marker-positive cancer cells have a growth advantage in the metastatic lesions.

Conclusions: HPC marker-positive cancer cells are more important for vascular invasion and metastasis than HPC negative cancer cells and suggested that HPC marker-positive cancer cells are an important target for HCC treatment.

P-0557

Functional role of TP53INP1 in HCC metastasis

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Background: The recurrence and metastasis of hepatocellular carcinoma (HCC) portends a poor prognosis and represents important

clinical challenges. There is a great need to identify critical factors involved in HCC metastasis that will facilitate the development of new therapeutic strategies. We have recently reported tumor protein 53 inducible nuclear protein 1 (TP53INP1) to be frequently down-regulated and to exert a tumor suppressive role in HCC (Ma et al. Cell Stem Cell 2010). In this study, we examined the role of TP53INP1 in HCC metastasis.

Methods: In vitro and in vivo functional studies using HCC cells edited by lentiviral based knockdown strategy were carried out to evaluate the effects of TP53INP1 on malignant phenotypes of HCC cells. A phospho-kinase array profiling was subsequently utilized to identify potential molecular mechanism that facilitates TP53INP1-mediated HCC metastasis.

Results: Stable knockdown of TP53INP1 in HCC promoted migration and invasion in vitro. Consistently, HCC cells with TP53INP1 silenced enhanced the cells' ability to metastasize to the lung from the liver in vivo. Proteome phospho-kinase profiling of HCC cells with or without TP53INP1 repressed identified significantly elevated levels of phospho-ERK1/2. Importance of ERK activation in TP53INP1-mediated HCC was further substantiated with rescue experiments using an ERK inhibitor U0126.

Conclusions: TP53INP1 down-regulation promotes metastasis in HCC through activation of ERK signaling.

P-0558

Functional role of Caveolin-1-mediated S100P signaling in liver cancer metastasis under hypoxia

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Hypoxia is a common feature of hepatocellular carcinoma (HCC) and facilitates tumor metastasis. High level of Caveolin-1 (Cav1) was exclusively expressed in primary HCC and metastatic tissues and its level was further enhanced under hypoxic stress. Suppression of Cav1 reduced HCC growth and metastasis. However, the potential role of Cav1 in human cancers under hypoxic stress has never been reported. A cDNA array profiling identified S100P calcium binding protein as a downstream target of Cav1. S100P is highly expressed in several malignancies and promote metastasis. In the present study, we showed that S100P expression was well correlated with Cav1 expression in a panel of HCC cell lines. In addition, S100P expression at protein and mRNA levels was largely suppressed in sh-Cav1 knockdown cells. Suppression of S100P expression largely inhibited tumorigenesis in subcutaneous injection and orthotopic liver implantation models. Furthermore, hypoxia significantly enhanced the migration and invasion of control and such enhancement was not prominent when Cav1 or S100P was suppressed. Taking together, Cav1 functions as the upstream component of S100P and plays a critical role in modulation of cell motility during hypoxia. Thereafter, our data showed Cav1 was able to activate NF- κ B reporter and transient expression of Cav1 upregulated S100P in cells. Addition of NF- κ B inhibitor, IMD-0354 abolished the upregulation of S100P by Cav1 suggests that Cav1 upregulates S100P via the activation of NF- κ B pathway. In conclusion, Cav1 may upregulate S100P through the activation of NF- κ B cascade to confer the enhanced aggressiveness of HCC cells under hypoxic condition.

P-0559

Nuclear met-derived exosomes promote HCC metastasis and formation of lung premetastatic niche

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Met receptor tyrosine kinase triggers a wide range of normal physiological signaling cascades. However, aberration activation of the Met is commonly found in human cancers. Emerging evidence has shown that nuclear Met (nMet) is expressed in some cancerous tissues and cell lines, suggesting nMet could have unexplored functions in the nucleus. Our previous studies have provided the first evidence about the clinical relevance of nMet in human hepatocellular carcinoma (HCC). Functionally, nMet promoted HCC tumorigenesis and metastasis. In our continual study to elucidate the underlying mechanism of nMet in driving HCC metastasis, we explored the significance of exosomes in HCC. In our present study, exosomes were isolated from metastatic MHCC97L HCC cell in which nMet was overexpressed. Electron microscopy images of the exosomes isolated from the conditioned media of control MHCC97L/Vec and MHCC97L/nMet cells showed typical exosome structure with diameter of approximately 50–80 nm. Immunoblotting showed that the isolated exosomes were highly enriched in exosomal markers while depleted of the cytoskeletal protein beta-tubulin, cis-Golgi marker GM130 and nucleoporin p62. Functional assays showed that exosomes derived from MHCC97L/nMet cells significantly augmented both the migratory and invasive properties of normal liver and naïve HCC cells. The uptake of PKH26-labeled nMet-exosomes by naïve cells were investigated by fluorescence microscopy. In animal model, intravenous injection of nMet-exosomes enhanced the incidence of distant metastasis from HCC primary tumor to lungs. Our findings will provide an important paradigm about tumor-derived exosomes in driving metastasis and yield novel mechanistic insights into liver cancer metastasis.

P-0560

Preventive effects of pentoxifylline on liver tumorigenesis in a NASH-related liver cancer model

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Non-alcoholic steatohepatitis (NASH) and NASH-related liver cancer have received attention. Although no standard medicinal treatment for NASH is established, pentoxifylline (PTX), a medicine used as a circulation improver, is reported to improve histological appearance of NASH. In the present study, we investigated effects of PTX on NASH and the development of diethylnitrosamine (DEN)-induced liver tumorigenesis in monosodium glutamate (MSG)-treated ICR mice, a novel model of NASH-related liver cancer, and db/db obese mice. Mice were administered DEN, and then they received water

with or without PTX (100 mg/kg/day). At sacrifice, the development of liver tumor was significantly inhibited in PTX group. MSG mice were thought to be susceptible to hepatocarcinogenesis compared to normal ICR mice. Hepatic steatosis and triglyceride accumulation in the liver were suppressed by PTX treatment. The serum levels of triglyceride, free fatty acid and alanine aminotransferase were all decreased by PTX, which also decreased mRNA expressions of TNF- α , IL-1 β , and several genes related to lipogenesis in the liver. The altered expression levels of those genes were also observed in *in vitro* study using cell lines treated by PTX. These findings suggest that PTX prevents NASH-related liver tumorigenesis by attenuating chronic hepatic inflammation and decreasing lipogenic gene expressions in the liver. PTX may be a potent agent for NASH patients who are at risk for developing liver cancer.

P-0561

Characterization of circulating and intrahepatic NK, NKT, CD4+ and CD8+ T cells in HBV-related HCC

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Background: To characterize the percentage of T cell subsets, NK, NKT and PD-1 expression on those cells in peripheral blood and liver tissues of HBV-related hepatocellular carcinoma (HCC) patients and to investigate their possible roles in HCC immunopathogenesis.

Methods: Twenty-five patients with HBV-related HCC and 21 normal controls were enrolled. Peripheral blood, tumor tissues, adjacent non-carcinoma tissues (<2 cm from HCC tissues), surrounding non-carcinoma tissues (>2 cm from HCC tissues) and normal liver samples were collected for the detection of T cell subsets and PD-1 expression on CD4+ and CD8+ T cells, NK cells and NKT cells by flow cytometry.

Results: In the peripheral blood, the percentages of CD4+ T cells, CD8+ T cells and the expression of PD-1 on T cell subsets, CD56brightNK cells, CD56dimNK cells and NKT cells as well as the ratio of CD4+/CD8+ and CD56bright/CD56dim between HBV-related HCC patients and normal controls had no significant difference ($P > 0.05$). However, the percentage of CD8+ T cells, CD56dimNK cells and NKT cells in tumor tissues significantly decreased compared to adjacent non-carcinoma tissues, surrounding non-carcinoma tissues and normal controls ($P < 0.05$). While the CD4+ T cells, CD4+/CD8+ ratio and PD-1 on CD8+ T cells were significantly increased in the HCC tissues ($P < 0.05$).

Conclusion: Aberrant infiltration of T cell subsets, NK, NKT and accumulation of exhausted PD-1 positive CD8+ T cells were observed in the HBV-related HCC tissues, implicating immunosuppressive tumor microenvironment and PD-1 might be potential target for immunotherapy for HBV-related HCC.

P-0562

Fusion HBx from HBV integrant might affect ER stress response and be associated with HCC

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Background/objectives: Hepatitis B virus (HBV) is a major risks factor associated with hepatocellular carcinoma (HCC), and HBV integration has been suggested to be associated with hepatocarcinogenesis. However, its molecular mechanisms remains unclear. In this study, we identified fusion HBx from HBV integrant in human hepatoma cell line, and investigated its role in hepatocarcinogenesis.

Methods: (1) We identified fusion HBx translated from HBV integrant in Hep3B cells, which consisted of 3'-truncated HBx following 61 amino acids from human sequences, and established stably knocked-down (KD) cells against fusion HBx by siRNA. (2) Using KD cells, we examined the effect of fusion HBx on cell growth, invasion ability and tumorigenicity *in vivo*. (3) We examined the expression change of mRNAs in KD cells using microarray, and gene set enrichment analysis (GSEA) was performed to investigate the signature of fusion HBx.

Results: (1) We established KD cells in which fusion HBx was disappeared by immunofluorescence. (2) In KD cells, cell proliferation and invasion ability was reduced. In addition, KD cells could not develop any visible tumor in nude mice when we injected KD cells subcutaneously into nude mice. (3) We identified 305 up-regulated and 115 down-regulated genes in KD cells by more than two folds. In GSEA, up-regulated genes in KD cells were enriched in endoplasmic reticulum (ER) stress response.

Conclusions: Fusion HBx translated from HBV integrant might affect ER stress response and play an important role in hepatocarcinogenesis.

P-0563

NASH-related hepatocarcinogenesis is suppressed in mice lacking hepatic retinoid storage

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Non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH), which are highly associated with metabolic syndrome, can lead to liver cirrhosis and hepatocellular carcinoma (HCC). Although hepatic retinoid (vitamin A) stores are progressively lost during the development of liver diseases, how this affects NAFLD/NASH and NASH-related hepatocarcinogenesis is unknown.

In order to investigate this, we used streptozotocin (STZ) and high-fat diet (HFD) to induce NASH and related hepatic tumorigenesis in matched wild-type and lecithin:retinol acyltransferase (LRAT) knockout (KO) mice, which lack stored retinoid in the liver. LRAT is the sole enzyme responsible for hepatic retinyl ester synthesis since LRAT KO mice completely lack lipid droplets in hepatic stellate cells. Mice were injected with STZ within 48 h after birth and then fed HFD from 4 to 16 weeks of age. At the termination of the experiment, liver tumors were observed macroscopically and microscopically in both groups. In LRAT KO mice, the development of hepatic adenoma and HCC was significantly suppressed compared to control group. LRAT KO mice showed decreased expression levels of cyclin D1 mRNA in the liver. The serum levels of d-ROMs and BAP were measured and the d-ROM/BAP ratio, which indicates oxidative stress, markedly decreased in LRAT KO mice. These findings indicate that LRAT KO mice are less susceptible to STZ and HFD-induced liver tumorigenesis due to regulation of cell cycle and attenuation of oxidative stress. The amount of hepatic retinoid may affect the progression of NASH and the development of NASH-related HCC.

P-0564

Hepatitis C virus might regulate suppressor of cytokine signaling-1 gene methylation to tumorigenesis

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Aim: To study HCV influence to suppressor of cytokine signaling (SOCS)-1, a tumor suppressor, we detected SOCS-1 expression in HCV related hepatocellular carcinoma (HCV-HCC) related tissues, and analyzed correlation between HCV and SOCS-1 in tissues and HCV replicon. Epigenetic regulation was also studied for HCV tumorigenesis.

Methods: HCV-HCC tissues, adjacent tissues, distal tissues and normal liver tissues were collected. SOCS-1 expression in tissue sections was detected by immunohistochemistry. After quantifying viral load, correlation between SOCS-1 and viral load was analyzed in tissues. Replicon was used to detect HCV influence to SOCS-1 afterwards. Then methylation specific PCR was used to learn the methylation status of SOCS-1 genes in tissues. After that correlation between gene methylation, SOCS-1 expression and viral load were analyzed.

Result: We collected 10 HCV-HCC tissues, 7 adjacent tissues, 7 distal tissues and 16 normal liver tissues. SOCS-1 expression showed tendency as HCV-HCC tissues < normal liver tissues < HCV-HCC adjacent/distal tissues. When comparing tissues with different viral load, higher viral load group showed lower SOCS-1 expression ($P = 0.0418$). Similarly, expression of SOCS-1 mRNA and protein were lower in replicon cells. SOCS-1 gene methylation was found in all HCV infected tissues but higher in HCC tissues ($p < 0.0001$). It showed positive correlation with viral load ($r = 0.5532$, $P = 0.0262$), implying HCV involvement in gene methylation.

Conclusion: HCV-HCC tissues had low SOCS-1 expression resulting from HCV. The low SOCS-1 expression in HCV-HCC tissues was related to high gene methylation. HCV might be involved in the regulation of such mechanism.

P-0565

C-terminal truncated hepatitis B virus × protein regulates stemness via Stat3/Nanog pathway

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Hepatitis B virus (HBV) is a major risk factor of chronic liver disease and hepatocellular carcinoma (HCC). Random integration of HBV DNA into the host genome is frequent in HCC leading to truncation of the HBV DNA, particularly at the C-terminal end of the HBV × protein (HBx). C-terminally truncated HBx (HBxΔC) has been implicated in playing a pro-oncogenic role in hepatocarcinogenesis and metastasis. However, its role on cancer stemness of HCC remains unclear. Thus, the functional role of HBxΔC regulating cancer stem cell (CSC) properties in HCC was investigated. Using Tet-on over-expression system, we found that HBxΔC enhanced CSC properties, including self-renewal, chemoresistance, tumorigenicity, and expression of CSC markers, when compared with transfectants derived from full-length HBx and vector control. In addition, a number of stemness genes were upregulated upon ectopic transfection of HBxΔC in which Nanog was found to be most prominent. Further study revealed that HBxΔC-induced CSC genesis through Stat3–Nanog pathway, as evidenced by the abolishment of self-renewal capacity induced by HBxΔC upon administration of small molecule inhibitor against STAT3. Consistently, Nanog was also significantly upregulated in HCC tissues with HBxΔC compared to those with full length HBx and non-HBV cases. Our data suggests that C-terminally truncated HBx regulates cancer stemness through STAT3–Nanog pathway, which also provides a new therapeutic target against HCC.

P-0566

Preventive effect of geraniol on hepatocarcinogenesis in diethylnitrosamine-induced rats

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Aims: The anti-carcinogenic effects of geraniol were investigated in the diethylnitrosamine (DEN)-induced hepatocarcinogenic rat models.

Methods: The male Wistar rats were intraperitoneally injected with 300 μl PBS [Group 1 (G1); n = 4] or DEN (100 mg/kg body weight) dissolved in PBS [Group 2 (G2); n = 8] every 2 weeks on experimental weeks 2, 4 and 6. The rats were treated with 0.07 % geraniol [Group 3 (G3); n = 9] and 0.35 % geraniol [Group 4 (G4); n = 7] for 12 weeks. We compared the relative liver weight (liver weight/body weight), serum AST, ALT, and ALP levels, and expression levels of proliferating cell nuclear antigen (PCNA) and glutathione S transferase-P (GST-P) by immunohistochemical analysis between each group.

Results: Relative liver weight was significantly higher in G2 than in G1 ($P < 0.01$). Both serum AST and ALT levels were significantly higher in G2 than in G3 and in G4 ($p < 0.05$). Serum ALP levels did not show a significant difference between each group. Percentages of both PCNA- and GST-P- positive cells, which are hepatocarcinogenic markers, were significantly decreased in G3 and in G4 compared to in

G2 ($P < 0.0002$ and $P < 0.0005$, respectively), revealing anti-hepatocarcinogenic effects of geraniol.

Conclusion: Geraniol is a promising compound useful for suppression of hepatocellular carcinoma. The mechanisms of action need to be clarified in the future.

P-0567

The significance of immune cells in peripheral blood with hepatocellular carcinoma

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Background: Immune cells, such as cytotoxic T-cells (CTLs), regulatory T cells (Treg), NK cells or dendritic cells (DCs), have been suggested to be associated with the progression of chronic liver diseases and development of hepatocellular carcinoma (HCC). Our objective is to evaluate whether those immune cells are associated with the prognosis of HCC.

Methods: We firstly examined the proportion of immune cells using peripheral blood with 149 patients with chronic liver diseases. Ninety-three patients had HCC whereas 56 patients had no-HCC. We defined CTL, myeloid DC, exhaust CTL and Treg as CD8+ CD69+ , Lineage-DR+ CD11c+ , PD1+ CD8+ and CD4+ CD25+ FoxP3+ , respectively. And then we followed those patients and checked their prognosis.

Results: As the results, myeloid DCs were decreased, and exhaust CTLs and NK cells were increased in patients with HCC, as compared with non-HCC (HCC vs non-HCC, 0.44 vs 0.45 %, $P = 0.05$, 0.60 vs 0.17 %, $P = 0.03$, 23.0 vs 16.4 %, $p < 0.01$, respectively). On the other hand, CTL and Treg were similar in both groups. Median survival time was 40 months, and the survival curve was classified as HCC stage developing. When we divided patients with HCC into two groups of the group which was higher than the median and a low group and compared them, only low myeloid DC group showed worse survival (myeloid DC 0.37 % < vs >0.37 %: 70 vs 32 months, $P = 0.004$).

Conclusions: Myeloid DCs are suggested to be associated with not only HCC development but also the prognosis. Exhaust CTLs and NK cells are suggested to be associated with HCC development alone.

P-0568

Endostatin gene delivery after irreversible electroporation for hepatocellular carcinoma

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Anti-angiogenic therapy had been considered to be a potentially effective strategy for hepatocellular carcinoma (HCC). Because of the short duration of most anti-angiogenic agents such as angiostatin or endostatin, the clinical value has been not so much. In this study, we examined the therapeutic effect of the endostatin gene delivered after irreversible electroporation (IRE) on implanted hepatic tumor. GP7TB cells were injected to the liver left lateral lobe of Fischer rats to induce a hepatic tumor. The different size and stage of tumors will

be obtained when laparotomy performed on the day 1st, day 7th and day 14th. The therapeutic strategy is to inject human endostatin plasmid into the tumors, and then IRE was applied over the tumors. The tumors were harvested 21 days later after tumor implantation. Tumor size was measured, and the microvessel density, apoptosis and the differentiation of these neoplastic lesions were investigated, too. The complete ablation rate was increased significantly on the rats receiving endostatin injection compared to those receiving placebo only or without followed IRE. The antiangiogenesis was also inhibited in these animals. The combination of endostatin gene delivery and IRE maybe will be a useful method to improve the therapeutic efficacy of HCC.

P-0569

The role of cellular immunity in the pathogenesis and treatment of patients with liver cancer

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The issues of pathogenesis of Liver Cancer (HCC) have not been fully understood so far. The arsenal of medications for treatment of this disease has also been scarce. The goal of our research was to study the results of cellular immunity activation in treatment of advanced cases of HCC. 22 patients with advanced stages of HCC which were not associated with viral hepatitis were used for observation. The patients received treatment according to the protocol of Doxorubicin and Cyclophosphamide combined therapy. In order to activate the cellular immunity, we utilized biological concentrate Zell Oxygen Immunkomplex (by Dr. Wolz) and Phytohaemagglutinin. The above substances (solutions) were given to the patients according to the certain dosage regimen and the previously selected schedule (per os) for 3 months. The control group comprised of 15 patients with the advanced form of liver cancer which received treatment only by Doxorubicin and Cyclophosphamide combined therapy protocol. The values of the cellular immunity were assessed by means of Immunocyto-chemical research of blood lymphocytes: CD3+ , CD4+ and CD8+ quantitative indicators of the cells. The results of the study have shown that: (a) before treatment, the cellular immunity of all the patients was suppressed by equal index; (b) in 3 months after treatment, cellular immunity of the observed patients increased by 29 % against the control group. With the results of the study, we can conclude that the activation of cellular immunity plays the essential role in the regimen of the combined therapy of HCC.

P-0570

PI3 K/Akt/mTOR signaling pathway mediates hepatoma cell autophagy induced by hydrogen sulfide

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Hydrogen sulfide (H2S) plays an important role in the pathogenesis of liver diseases. However, the role of H2S in pathogenesis of

hepatocellular carcinoma (HCC) remains unclear. Here we used NaHS, an exogenous H2S donor, to evaluate the role of H2S in autophagy in HepG2 and HLE cells. After treatment with NaHS, the percentage of cells with LC3 punctate dots was clearly increased in HepG2 ($64.7 \pm 3.15\%$ vs $8.77 \pm 0.89\%$, $P < 0.05$) and HLE ($47.87 \pm 6.93\%$ vs $7.0 \pm 0.52\%$, $P < 0.05$). The intracellular double-membrane vesicles in HepG2 and HLE cells were increased. Furthermore, the autophagy related proteins: Beclin 1, Atg5 and LC3-II were up-regulated and beclin1 mRNA, atg5 mRNA levels were also increased after treatment using NaHS. The p-PI3 K, p-Akt, and mTOR proteins were reduced. Notably, the autophagy and LC3-II were more increased when the cells were treated using rapamycin, the mTOR inhibitor. Taken together, these findings reveal that exogenous H2S can promote hepatoma cell autophagy by the inhibition of PI3 K/Akt/mTOR signaling pathway.

P-0571

Hepatitis B virus remains the leading cause of HCC in Bangladesh

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Aim: Our study presented at Hong Kong APASL 2009 showed that HBV is the leading cause of HCC in Bangladesh. Aim of present study was to see if there is any change in the etiology of HCC in this country 6 years from Hong Kong.

Methods: This is a retrospective study. Patients admitted in Hepatology Green Unit, Bangabandhu Sheikh Mujib Medical University, Dhaka in 2014 with HCC were included.

Results: Total 429 patients were admitted. Of them 40 (9.3 %) were diagnosed with HCC. Diagnosis was made on the basis of imaging, serum AFP and/or cytology. Of them 52.5 % [21/40] cases were due to HBV and 12.5 % [5/40] due to HCV. However in 35 % [14/40] the aetiology was NASH. Although HBV remains the leading cause of HCC in Bangladesh the burden has declined significantly (75 % in 2007). On the other hand, NASH (5 % in 2007) has become much more important bypassing HCV (17 % in 2007) as the second leading etiology of HCC in Bangladesh.

Conclusion: HBV is the commonest cause of HCC in Bangladesh. Our government has integrated HBV vaccine into the existing Expanded Programme of Immunization. However we have to go a long way before we can sustain HBV and its complications in Bangladesh. Besides NASH is also becoming a concern.

P-0572

Heterogeneity among Asians with hepatocellular carcinoma (HCC): a single center study in California

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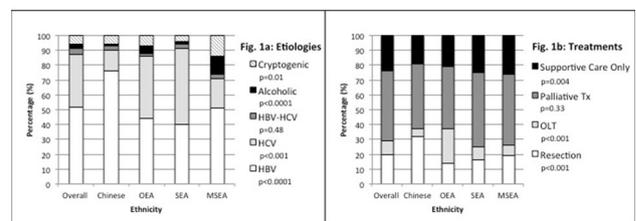
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Background: Asians have the highest HCC incidence in the U.S. We evaluated differences in etiology, presentation, treatment and survival among 5 Asian sub-groups.

Methods: This was a retrospective cohort study of 824 consecutive Asian HCC patients seen at a university medical center from 1993 to 2015, subdivided as: Chinese (n = 252), Other East Asian (OEA, n = 84; Japanese and Korean), South East Asian (SEA, n = 389), Maritime South East Asian (MSEA, n = 69; Malaysian, Indonesian, Filipino, and Singaporean), and South West Asian (SWA, n = 30; Indian, Pakistani, and Middle Eastern).

Results: The majority of patients were male (76 %) with mean age of 63. HCC etiology and presentation varied between sub-groups (Fig. 1a). Chinese patients were more likely to have HBV, OEAs and SEAs HCV, and MSEAs cryptogenic cirrhosis ($p < 0.05$). Chinese patients had the lowest prevalence of cirrhosis (58 % vs 75–87 %, $p < 0.001$), while MSEAs were more symptomatic on presentation (44 % vs 32–58 %; $P = 0.07$). OEAs were more likely to have tumors ≤ 5 cm (21 % vs 36–48 %; $P = 0.001$) and to meet Milan criteria (63 % vs 35–45 %). Along with Chinese patients, they received more primary surgical interventions, especially transplantation (Fig. 1b). MSEA and SWA patients trended towards lower 10-year survival (9 and 18 % respectively vs 32 % Chinese, 30 % SEA, and 25 % SEA patients; $P = 0.07$). Multivariate analysis demonstrated that older age, higher tumor stage, and treatment modality (surgical vs. palliative) significantly altered survival, but not ethnicity or etiology ($p < 0.05$)

Figure 1: Prevalence of HCC etiology and treatments among Asian American sub-groups



Conclusion: Great heterogeneity exists among Asian HCC patients. Culturally targeted interventions are necessary to help prevent and diagnose HCC early.

P-0573

Survival rate of HCC patients after fifteen years in a tertiary referral hospital in Indonesia

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Background: In 1998–1999, the survival of hepatocellular carcinoma (HCC) patients in Cipto Mangunkusumo Hospital as a tertiary referral hospital, was very poor because most patients came in advanced stage and only few patients could receive palliative or curative treatment. For the last 3 years, HCC treatment facilities have been improved. It is unclear whether this effort has resulted in improvement of patients' survival.

Methods: We analyzed retrospectively 114 HCC patients who came to our department in 2013–2014 and 77 patients in 1998–1999. The

clinical characteristics and treatment between two periods were compared and we analyzed the survival of both groups using Kaplan–Meier method and compared them using log-rank test.

Results: There was an increase in hepatitis B prevalence as the etiology of HCC from 32.5 % in 1998–1999 to 67.5 % in 2013–2014, causing hepatitis B as the main etiology of HCC in 2013–2014. Incidence rate of patients who died in 2013–2014 was 57 % [95 % confidence interval (CI) 48–66 %] and in 1998–1999 was 61 % (95 % CI 49–73 %). Overall median survival was 141 days. Despite improvement in treatment facilities, no significant difference was found in one-year survival rate (29.4 % in 2013–2014 vs. 24.1 % in 1998–1999, $P = 0.913$). It seems that this result was caused by low level of surveillance in high-risk population.

Conclusion: No improvement was seen in one-year survival rate of HCC patients between 2013–2014 and 1998–1999. Key words: hepatocellular carcinoma, survival, time period.

P-0574

Aflatoxin B1 as a preventable risk factor in the development of HCC in Egyptian patients

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Background: Aflatoxin is among the most important environmental toxins contributing to the pathogenesis of HCC. Aflatoxin B1 (AFB1) is the most hepatotoxic and hepatocarcinogenic agent. Individuals continuously exposed to this toxin through contaminated food grains and animal products may develop both acute hepatotoxicity and HCC particularly in many developing countries. This study investigates the possible additive role of AFB1 exposure in the development of HCC in Egyptian patients in Nile Delta.

Methods: From January 2014 to July 2014, a total of 40 cirrhotic patients with HCC and another 30 cirrhotics without HCC were selected from Tanta Liver Center, and 10 healthy controls. AFB1 albumin adduct in serum was estimated in all subjects using enzyme-linked immunosorbent assay.

Results: 30 (75 %) of the 40 HCC patients had viral related and 10 (25 %) had non-viral etiology. 29 (96.4 %) of viral related HCC were HCV-related and only one HBV-related (3.3 %). Of the 30 cirrhotics without HCC, 23 (76.7 %) had viral etiology, and all were due to HCV infection (100 %). AFB1 was detected in the serum of all HCC patients whether of viral or non-viral etiology (100 %), and in 27 (90 %) of cirrhotics without HCC.

Conclusions: The coexistence of AFB1 albumin adduct, a reliable biomarker of AFB1 exposure, along with HCV in 96.7 % of viral-related HCC is indicative of its additive hepatocarcinogenic action. The presence of AFB1 in serum of all non-viral HCC is indicative of a possible primary hepatocarcinogenic effect. Our study emphasizes the need to develop strategies to reduce mycotoxin exposure in Egypt.

P-0575

Prevalence and risk factors of hepatocellular carcinoma in Egyptian cirrhotic patients

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Aim of the study is to detect the prevalence and risk factors of HCC in Egyptian cirrhotic patients.

Patients and methods: The study included 1514 patients with liver cirrhosis from Menoufia University Hospitals (Egypt). They underwent physical examination and laboratory investigations (HCV Ab, HBsAg, HCV PCR and AFP). Abdominal ultrasonography was done for all patients. HCC is further confirmed by triphasic CT.

Results: Out of the 1514 examined cirrhotic patients, 302 patients (19.9 %) had HCC. HCC cases ($n = 302$) are significantly older than cirrhotic patients without HCC ($n = 1212$) ($P = 0.01$). Smoking is significantly more prevalent in HCC cases (83.4 %) than cirrhotic patients without HCC (50.5 %) ($P = 0.0001$). The prevalence of HBsAg and HCVAb was significantly higher in HCC cases (12.9 and 87.1 %) than cirrhotic patients without HCC (5.9 and 77.7 %) ($P = 0.01$). On multivariate regression analysis, risk of HCC development was 4.4 times more in smokers than nonsmokers ($P = 0.001$), around 3.5 times more in HBsAg positive cirrhotic patients than HBsAg negative cirrhotic patients and 3.4 times more in HCVAb positive cirrhotic patients than HCVAb negative cirrhotic patients.

Conclusion: Egypt has a high incidence of HCC about 20 % in cirrhotic Egyptian patients. HCV and HBV infections and smoking are the main determinants of HCC development in Egyptian cirrhotic patients. An active surveillance and secondary prevention programs for patients with chronic hepatitis are the most important steps to reduce the risk of HCC.

P-0576

HCC burden in Egypt

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Aim: Prevalence and risk factors of Hepatocellular carcinoma (HCC) in cirrhotic Egyptian patients. HCC is the commonest cancer of the liver. The burden of HCC has been increasing in Egypt. Our survey on 1350 Egyptian cirrhotic patients revealed that 289 patients had HCC (21.4 %). Our study about risk factors of HCC on cirrhotic Egyptian patients was done on 300 patients with HCC and 50 patients with chronic liver diseases without HCC (controls). Risk of HCC development in smokers with HBV or HCV was 4.90 and 8.47 (OR) ($P = 0.0001$). It was higher than in non-smokers with HBV or HCV (OR = 2.48 and 4.44) ($P = 0.037$ and 0.0001) and in smokers without HBV or HCV (OR = 2.56 and 2.77) ($P = 0.01$). The risk of HCC development in HBV or HCV positive patients with DM was 3.98 and 9.19 (OR) ($P = 0.001$ and 0.0001). It was higher than for HBV or HCV positive patients without DM (OR = 2.8 and 4.65) ($P = 0.031$ and 0.0001) and that for HBV or HCV negative patients with DM (OR = 2.56 and 2.23) ($P = 0.01$ and 0.0001). Our study about the prevalence of occult HBV (OBI) among HCV positive Egyptian patients documented that out of 100 chronic HCV patients; only 16 patients (16 %) had OBI. HCC was significantly more common in OBI/HCV dually infected (31 %) than HCV monoinfected patients (7 %) ($P = 0.01$). On doing multiple logistic regression analysis, OBI is a risk factor for HCC in Egyptian HCV positive patients ($P = 0.04$).

Conclusion: Egypt has a high incidence of HCC about 21 % in cirrhotic Egyptian patients. HCV and HBV infections, diabetes and smoking are the main determinants of HCC development in Egypt. Occult HBV influence the outcome of HCV infection leading to development of HCC.

P-0577

Relationship between HCC and serum markers in NA-treated patients with undetectable HBV DNA**Ka Shing Cheung, Wai Kay Seto, Danny Ka Ho Wong, Ching Lung Lai, Man Fung Yuen**

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Background and aims: Patients with undetectable HBV DNA under nucleos(t)ide analogue (NA) treatment can still develop hepatocellular carcinoma (HCC). We aimed to examine the relationship between hepatitis B surface and core-related antigens (HBsAg; HBcrAg) and HCC development.

Methods: Seventy-six HBV carriers who developed HCC with undetectable serum HBV DNA after at least one-year NA therapy were compared with 152 matched controls. Clinical and laboratory parameters were analysed.

Results: There were no significant differences in HBsAg or HQ-HBsAg levels. There was a significant difference in the median values of both pre- and post-treatment HBcrAg levels between the HCC group and controls (pre-treatment: 279.0 vs 35.4 kU/mL, respectively, $P = 0.005$; post-treatment: 10.2 vs 1.7 kU/mL, respectively, $P = 0.005$). A cutoff value of post-treatment HBcrAg level >7.8 kU/mL yielded an area under receiver operating curve (AUROC) of 0.61 with a negative predictive value (NPV) of 77.0 %. The OR of HCC development was 3.27. For the subgroup of non-cirrhotic patients, the median values of post-treatment HBcrAg level of the HCC group and controls were 10.2 and 1.0 kU/mL respectively ($P = 0.001$). A cutoff value of HBcrAg level >7.9 kU/mL yielded an AUROC of 0.70 with a NPV of 80.6 %. The OR of HCC development was 5.95.

Conclusion: A higher pre- and post-treatment HBcrAg level (but not HBsAg or HQ-HBsAg) was associated with an increased risk of HCC development in patients who achieved undetectable serum HBV DNA while on NA therapy. The effects of NA on HBcrAg level for reduction of HCC development warrant further investigation.

P-0578

Role of serum glypican-3 in the diagnosis of small hepatocellular carcinoma**Tarek Korah, Eman Badr, Ashraf Abdel Ghani, Sawsan El-Sayed, Safaa Badr**

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This study addressed the role of serum glypican-3 (GLP-3) in the diagnosis and differentiation of small hepatocellular carcinoma (HCC) from hepatitis-C virus (HCV) cirrhosis, and included discussion of serum GLP-3 levels in the early diagnosis and differentiation of small (3 cm or less in diameter) HCC from liver cirrhosis, and correlation of GLP-3 levels to clinico-laboratory data. This study included sixty patients; 30 of them with HCV cirrhosis, and 30 patients with proved HCC. Also, 20 healthy subjects as a control group. Clinical and radiological features (abdominal ultrasonography and/or abdominal triphasic computed tomography) were recorded. Liver function tests, complete blood cell count, and serum AFP were measured. Serum GLP-3 values were determined by an ELISA technique. In addition, attendees of this lecture will note the conclusions of this topic, which include that serum GLP-3 levels are higher in HCC versus HCV cirrhosis, that can differentiate HCC from

liver cirrhosis. Also, serum GLP-3 is highly sensitive and specific for detecting HCC. Moreover, GLP-3 is more sensitive than AFP for the detection of small HCC. Furthermore, a combination of both serum markers yielded an improved specificity and both sensitivity and specificity for the diagnosis of small and unicentric HCC, respectively.

P-0579

Serum chitotriosidase levels in malignant diseases of liver: is it a new tumor marker?**Engin Altintas¹, Serkan Yaras¹, Mehmet Kasim Aydin¹, Mahmut Bakir Koyuncu¹, Enver Ucbilek¹, Burak Cimen², Fehmi Ates¹, Orhan Sezgin¹**¹Gastroenterology, Mersin University Faculty of Medicine, Yenisehir, Mersin, Turkey; ²Biochemistry, Mersin University, Faculty of Medicine, Mersin, Turkey

Aim: We found that serum chitotriosidase levels were significantly higher in HCV related liver diseases especially in patients with hepatocellular carcinoma (HCC). Based on these results, we aimed to study serum chitotriosidase levels in malignant liver diseases.

Material and method: 62 patients had HCC, 50 patients had liver metastasis due to primary malignancies and 37 patients were in control group (C group). Serum samples which were collected at the time of diagnosis were tested using ELISA kit. The biochemical and demographic data of patients were recorded.

Results: Serum chitotriosidase levels of the patients in C group were lower compared with other groups (metastasis group and HCC group) and this difference was found statistically significant ($p < 0.000353$). Mean serum chitotriosidase levels were 214.380 ng/ml (13.00–827.82 ng/ml) in C group, 439.575 ng/ml (13.86–4186.40 ng/ml) in metastasis group and 428.735 ng/ml (13.18–4005.87 ng/ml) in HCC group. Metastasis group and C group were compared with ROC analysis and cut-off point was determined as 311.8 ng/ml ($P = 0.0001$, 95 % CI sensitivity 72 %, specificity 75, 68 %). HCC group and C group were compared with ROC analysis and cut-off point was determined as 240.93 ng/ml ($p < 0.0001$, 95 % CI sensitivity 74.19 %, specificity 67.57 %) (95 % CI PPD 74.19 %, NPV 67.57 %). In HCC group, there was no significant association with other parameters.

Conclusion: Serum chitotriosidase can be a tumor marker to distinguish malignant liver diseases from benign liver diseases. It can be used as a screening test in HCC like AFP.

P-0580

Serum fibrosis biomarkers can predict presence of hepatocellular carcinoma in chronic hepatitis C**Mohamed El-Kassas¹, Mohamed Alborae², Shima Afify³, Mohamed Nour⁴**¹Department of Endemic Medicine and Hepatology, Helwan University, Cairo, Egypt; ²Department of Internal Medicine, Al-Azhar University, Cairo, Egypt; ³Internal Medicine Department, National Hepatology and Tropical Medicine Research Institute, Cairo, Egypt; ⁴Faculty of Public Health and Health Informatics, Umm Al Qura University, Makkah, Saudi Arabia

Introduction: Surrogate biomarkers predicting hepatic fibrosis and cirrhosis are gaining popularity in clinical practice because they are reproducible and accepted by patients. They were extensively studied in chronic liver diseases especially chronic hepatitis C (CHC). Whether biomarkers can predict the presence of hepatocellular carcinoma (HCC) in CHC patients is not yet established.

Aims and Methods: To test the ability of 5 biomarker scores (APRI, FIB-4, Fibro alpha, BRC and FRT)1-5 to predict presence of HCC in patients with CHC. A case control study was designed comparing two groups of CHC patients; Group I included 186 CHC patients and Group II included 98 CHC patients complicated by HCC. All patients were subjected to CBC, ALT, AST, total bilirubin, albumin, INR, alpha fetoprotein (AFP), ultrasound abdomen, tri-phasic CT scan of the liver for Group II patients. Biomarker scores were calculated according to their original publications.

Results: All studied non-invasive biomarker scores were able to predict presence of HCC with good performance and AUROCs (0.77–93, 95 % CI). Scores containing AFP (Fibro alpha, FRT and BRC) were able to differentiate early from advanced HCC (p values 0.005, 0.010 and 0.013 respectively). Scores not containing AFP (FIB-4 and APRI) were significantly correlated with splenomegaly (p values: 0.001 and 0.005 respectively). All scores were not able to differentiate between cases with unifocal or multifocal lesions.

Conclusion: Non-invasive biomarker scores of hepatic fibrosis are useful in predicting HCC, its stage and the presence of splenomegaly in CHC patients.

P-0581

Serum Interleukin-6 levels as a predictive marker in patients with hepatocellular carcinoma

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Background: We aimed to investigate the use of novel serum biomarkers for predicting the recurrence and survival of patients with hepatitis B virus (HBV)-related early hepatocellular carcinoma (HCC) after hepatic resection or radiofrequency ablation (RFA).

Methods: One hundred and five patients with HBV-related HCC, who fulfilled the Milan criteria without vascular invasion and underwent hepatic resection or RFA, were followed-up for a median duration of 52 months. Pretreatment serum concentrations of 16 cytokines including interleukin-6 (IL-6) were measured by using a Luminex 200 system. The measured serum cytokines and several clinical factors were analyzed retrospectively.

Results: Univariate analysis showed that patients with lower pretreatment serum levels of IL-10, IL-6, monocyte chemoattractant protein-1, and tumor necrosis factor- α had significantly shorter disease-free survival (DFS) than those with higher levels. Multivariate analysis revealed that a low serum IL-6 level [<33.00 pg/mL; hazard ratio (HR) = 5.39; 95 % confidence interval (CI) = 1.27–22.93; $P = 0.022$], low platelet count ($<100 \times 10^9/L$; HR = 2.23; 95 % CI 1.28–3.89; $P = 0.005$), and low serum albumin level (<3.5 g/L; HR = 2.26; 95 % CI 1.28–3.97; $P = 0.005$) had a negative prognostic impact on DFS.

Conclusion: A low serum IL-6 level is, in addition to low platelet count and low serum albumin level, an independent prognostic factor for DFS in patients with HBV-related early HCC who underwent hepatic resection or RFA with curative intention.

P-0582

AFP as predictor of survival rather than diagnostic marker in patients W/HCC (retrospective cohort)

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Introduction: AFP is a widely tested HCC biomarker. However, aspects linked to fluctuations made its performance suboptimal in diagnosing HCC. New biomarkers have been introduced (DCP and AFP-L3). Recently, AFP was proposed as predictor of survival and tumor recurrence after surgery, locoregional and systemic therapies. Our aim is to investigate the role of AFP as predictor of 1-year survival in HCC.

Methods: A retrospective cohort study of patients with HCC from January 2003 to September 2014 in Cardinal Santos Medical Center was done. Demographic data of patients with AFP levels of <10 ng/mL (Group I N = 134) and >10 ng/mL (Group II N = 302) determined and one-year survival from time of diagnosis up to time of death.

Results: Among 436 patients with HCC, both groups had CPC B (Group 1 n = 46; Group 2 n = 187), and Diabetes Mellitus (Group 1 n = 74; Group 2 n = 178). Tumor characteristics were majority single (Group 1 n = 100; Group 2 n = 212), with a size of <3 cm (Group 1 n = 84; Group 2 n = 158). Among the subjects, 280 patients survived 1 year (64.68 %), with a rate of 81 % in AFP <10 ng/ml (n = 134), and 58 % in AFP >10 ng/ml (n = 302) demonstrating a higher mortality rate in HCC patients with higher AFP levels.

Conclusion: The results of the study showed that there is a higher percentage of one-year survival in patients with AFP <10 ng/dL compared to AFP >10 ng/dL. Despite the inability of AFP levels to diagnose HCC, it may still be used as a predictor of one-year survival for these patients.

P-0583

The clinical significance of HINT2 expression in hepatocellular carcinoma

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The histidine triad nucleotide binding protein 2 (HINT2) gene encodes a histidine triad protein that acts as a mitochondrial apoptotic sensitizer in hepatocellular carcinoma (HCC). However, the clinical significance of HINT2 expression in patients with HCC is unknown. We investigated HINT2 mRNA expression in tumors and adjacent nontumor hepatic tissues from 106 HCC patients using quantitative real-time PCR. Association between the HINT2 mRNA expression level and clinical and pathological parameters, including recurrence-free survival (RFS) and overall survival (OS), were evaluated using appropriate statistical methods. HINT2 was down-regulated significantly ($p < 0.0001$) in the tumor versus matched HCC non-tumor hepatic tissue. And both Kaplan–Meier survival curve and multivariate analyses showed a relationship between HINT2 and RFS. In conclusion, a low expression level of HINT2 in HCC indicated aggressive tumor behavior and predicted a worse clinical outcome.

P-0584

Clinical significance of circulating endothelial progenitor cells in patients with liver cirrhosis

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Background: The role of circulating endothelial progenitor cells (EPCs) in patients with liver cirrhosis (LC) has seldom been investigated. This study was conducted to assess the clinical significance of circulating EPCs in patients with LC with or without hepatocellular carcinoma (HCC).

Methods: A blood sample was collected once from patients with LC alone (n = 34) or LC and HCC (n = 46) and healthy controls (n = 27) for assessing levels of EPCs and vascular endothelial growth factor (VEGF). Blood cells staining positive for CD34/CD133/KDR using flow cytometry were characterized as EPCs. Plasma VEGF was quantified by ELISA.

Results: The levels of CD34/KDR-positive EPCs, CD133/KDR-positive EPCs, and VEGF were higher in patients with LC or LC and HCC than in healthy controls (P = 0.017, p < 0.001 and p < 0.001, respectively). The levels of EPCs and VEGF did not show statistical difference according to Child-Turcotte-Pugh class. There was a moderately significant correlation between VEGF levels and HCC stage in HCC patients (P = 0.464, P = 0.001). Smoking, ascites, and portal vein thrombosis were independently related to lower levels of circulating CD34/KDR-positive EPCs, higher levels of CD133/KDR-positive EPCs, and higher levels of VEGF, respectively (P = 0.041, P = 0.023, and p < 0.001, respectively).

Conclusions: Circulating EPCs and plasma VEGF levels were higher in patients with LC ± HCC compared to healthy controls. The increase in circulating EPCs and VEGF may have a possible role in the development of complications, especially ascites and portal vein thrombosis, or in progression of HCC.

P-0585

AFP still is a valuable diagnostic and prognosis predicting biomarker in HBV infection-related HCC

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Use of serum alpha-fetoprotein (AFP) in clinical practices has been challenged, due to the lack of specificity and sensitivity. Here we conducted a retrospective study to evaluate the diagnostic and prognostic value of serum AFP among hepatocellular carcinoma (HCC) patients with pathogenic features taken into consideration. The cohort for this retrospective study comprised 318 cases of hepatitis and 731 cases of cirrhosis, as well as 796 HCC patients. The positive rate of patients in the three AFP intervals (>20, >200, >400 ng/ml) were 73.6, 56.2, 50.6 % among HBsAg positive HCC patients, while which dropped sharply to 43.5, 32.9, 29.4 % among those negative for HBsAg (P < 0.000). In addition, Kaplan–Meier curve analysis revealed that lower preoperative AFP level implicated a much higher overall survival rate. However, such prognosis predicting value was

only seen in those chronic HBV infection-related HCC patients, but not among the HCC patients etiologically irrelevant to HBV infection. We believe that serum AFP is of diagnosis and prognostic predicting value for HCC with chronic HBV infection, and strongly suggest use of serum AFP as a biomarker in China and southeast Asia where HBV infection are endemic.

P-0586

Proteomics analyses identified a phosphoprotein relevant to apoptosis resistance in human hepatoma

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Apoptosis resistance is one of the biological characteristics of cancer cells, and leads to escape from immune surveillance and brings the limited efficacy of chemotherapy. To identify the key molecules which account for apoptosis resistance of hepatoma, we perform integrated comprehensive analyses. Two-dimensional differential gel electrophoresis (2-D DIGE) was applied to identify the differentially expressed proteins and their phosphorylation profiles of human hepatoma cell lines in the presence or absence of H₂O₂, an apoptosis-inducing stimulation. Meanwhile, cDNA microarray analysis was performed to determine change of the gene expression in the same samples.

Among proteins which showed change in phosphorylation status, Nucleophosmin is one of the most prominently changed protein. Knockdown of NPM using NPM specific siRNA in hepatoma cells enhanced cell death in H₂O₂ stimulation. Functional disruption of NPM phosphorylation with stably transfection of mutated NPM in hepatoma cells resulted in their reduced apoptosis resistance. Network analysis indicated that miRNA × is associated with NPM phosphorylation via proteins such as PTEN and Cdk. Expression and phosphorylation of NPM were significantly increased in human hepatocellular carcinoma (HCC) tissues compared to the adjacent non-cancerous tissues (n = 23). Recurrence-free survival after surgical resection is significantly longer in HCC with lower expression of NPM phosphorylation group (n = 14) than higher expression group (n = 9).

These results indicates that apoptosis resistance of human HCC is, in part, accounted for phosphorylation of NPM and downstream signaling, which may be a novel therapeutic target for human HCC.

P-0587

Evaluation of the diagnostic value of plasma GP73 and AFP in HCC patients infected with HBV

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Objective: To investigate clinical value of Golgi Protein 73 (GP73) in Chinese HBV patients with hepatocellular carcinoma (HCC) and compare utility of GP73 and alpha-fetoprotein (AFP) assays on HCC diagnosis and stages.

Methods: 295 HBV-infected patients were divided into 11 groups: 26 mild chronic hepatitis (LCHB), 22 moderate CHB (MCHB), 29 SCHB (severe CHB); 30 decompensated liver cirrhosis (DLC), 22 compensated LC (CLC); 32 HCC1 (early prophase, single tumor ≤ 2 cm), 30 HCC2 (prophase, single tumor, > 2 cm ≤ 4 cm); 90 HCC3 (aggressive) consisting of 30 HCC3A (single tumor > 4 cm), 30 HCC3B (tumor number ≥ 2), 30 HCC3C (large vessel invasion); and 14 HCC4 (advanced, distant metastasis). Plasma GP73 (U/ml) and AFP (U/L) were detected by ELISA (INOVA Diagnostics) and electrochemiluminescence (RocheE601) respectively.

Results: Median GP73 was lowest in HCC1 (10.4) and increased with HCC tumor size, multiple tumors, large vessel invasion, and pulmonary metastasis ($p < 0.0001$ for HCC1-HCC4). Interestingly, the median GP73 HCC1 value was lower than MCHB ($p < 0.0001$), SCHB ($p < 0.0001$), CLC ($P = 0.0048$), or DLC ($P = 0.0054$). In contrast, the log₁₀AFP value for HCC1 was higher ($P = ns$) than CHB and LC groups. The increase in GP73 from HCC1 became significant at HCC3b ($p < 0.0001$), while for AFP log₁₀, significant change was not observed until HCC3c ($p < 0.0001$). Using cutoffs of 20 U/ml (GP73) and 7 U/L (AFP), sensitivity for HCC with GP73, AFP, and GP73 + AFP was 35.0, 71.8, and 78.5 % and specificity was 62.5, 63.3 and 46.7 %, respectively.

Conclusions: Combined measurement of GP73 and AFP increased sensitivity, but decreased specificity. High GP73 levels in SCHB and lower levels in HCC1, contrasts with high AFP levels in SCHB and HCC1, perhaps indicating different mechanisms. While both GP73 and AFP increased from HCC1, GP73 changes were significant earlier at HCC3b compared to HCC3c for AFP, suggesting possible prognostic use.

P-0588

Factors associated with a higher alpha-fetoprotein level in patients with hepatocellular carcinoma

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Aim: Baseline alpha-fetoprotein (AFP) level is an independent predictor of survival in patients with hepatocellular carcinoma (HCC). In this study, we aimed to investigate clinical and oncological features associated with high AFP level in patients with HCC.

Methods: Patients with HCC who were followed-up from 2001 to 2011 were included in the study. Diagnosis was established by histopathological and/or radiological criteria. We retrospectively reviewed demographic, clinical and laboratory data, etiology of underlying liver disease, imaging characteristics in patients with HCC. Barcelona Clinic Liver Cancer (BCLC) stage was determined at initial diagnosis. Factors associated with high AFP level were investigated by multiple linear regression analysis.

Results: 545 patients with HCC (440 male, mean age 59.5 ± 10 years) were included in the study. 454 patients had underlying chronic viral hepatitis. Baseline AFP level was 62 ng/ml (range 1–223169 ng/ml), and 194 (35.6 %) patients had AFP > 200 ng/ml. In patients with chronic viral hepatitis, AFP level was higher than in patients having other etiologies (71 vs 14 ng/ml, $P = 0.016$). BCLC stage C and D were found to be associated with a higher AFP level compared with BCLC stage 0–A ($P = 0.001$ and $p < 0.001$, respectively). Multivariate analysis showed that total tumor diameter, vascular invasion, diffuse-infiltrative HCC and viral etiology were independently associated with high AFP level ($R^2 = 0.156$, $F(12,410) = 8$, $p < 0.001$).

Conclusion: Underlying chronic viral hepatitis, tumor type, burden and extension are associated with a high AFP level.

P-0589

The impact of alpha fetoprotein (AFP) level during IFN free treatment for Hepatitis C

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Aim: Elderly patients are at a higher risk for HCC even after HCV eradication. AFP levels after IFN therapy for hepatitis C are significantly associated with hepatocarcinogenesis. However the meaning of AFP value after IFN free therapies for older patients remains unclear. To further clarify this, we analyzed the value of AFP during IFN-free treatment.

Patients and methods: This study included 63 patients (34 men, 29 women) with HCV genotype 1b who received daclatasvir 60 mg once daily plus asunaprevir 100 mg twice daily. The average of age of these patients is 70.7 years old (38–83 years). The level of AFP was periodically assessed at baseline and every month. These cases were followed up by abdominal ultrasonography (US), contrast computed tomography (CT) or magnetic resonance imaging (MRI) every 3–6 months.

Results: The serum average level of AFP of all patients before treatment was 26.3 ng/ml and tend to decrease to 11.5 ng/ml at the end of treatment. Serum AFP level in 31 patients reduced to less than 5 ng/ml at the end of treatment (Group A). Only one patient (3 %) of Group A diagnosed HCC. However 27 patients remained more than 5 ng/ml at the end of treatment (Group B) were examined for HCC and 13 patients (48 %) were diagnosed with HCC after the end of treatment.

Conclusion: AFP trend is useful for predicting future HCC risk during and after IFN free therapy. Hepatitis C patients with AFP level more than 5 ng/ml at the end of treatment should be examined for HCC with US, CT and/or MRI as soon as possible.

P-0590

The characteristics of hepatocellular carcinoma with elevated AFP-L3 level and normal AFP level

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Aim: α -fetoprotein (AFP) and lens culinaris agglutinin-reactive fraction of α -fetoprotein (AFP-L3) have been widely used as a tumor marker for the diagnosis and follow-up surveillance of hepatocellular carcinoma (HCC) in Japan. Elevated AFP-L3 level is considered to indicate poor prognosis for patients with HCC. However, the prognostic value of elevated AFP-L3 within normal AFP level in patients with HCC remains to be elucidated.

Methods: This retrospective study enrolled 242 patients with elevated AFP-L3 level (≥ 15 %) with AFP level within normal range (< 10 ng/mL) during routine HCC surveillance at our hospital. Of 242 patients, 26 patients were newly diagnosed HCC and 216 patients had previous history of HCC. We analyzed the presence of vascular invasion and extrahepatic metastasis in these patients.

Results: Of 242 patients, 19 patients had already had vascular invasion or extrahepatic metastasis before the study enrollment. Eight patients were newly diagnosed as vascular invasion ($N = 3$) or hepatic metastasis ($N = 5$) at study enrollment. The remaining 215 patients were not diagnosed as vascular invasion or extrahepatic metastasis at study enrollment. However, 21 patients developed vascular invasion or extrahepatic metastasis during the follow-up period (median 476 days).

Conclusion: Elevated AFP-L3 within normal AFP level may indicate or predict advanced HCC.

P-0591

Elevated preoperative serum CEA levels in patients with HCC is associated with prognosis and EMT

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Introduction: EMT plays an important role of tumor malignancy including metastasis and invasion, and the elevated tumor markers has been reported as a prognostic factor in several malignant tumor. In this study, we evaluated the significance of serum CEA levels for prognosis. In addition, increased serum CEA reflected Carcinoembryonic antigen-related adhesion molecule 1 (CEACAM1) expression, moreover related to EMT-related factors (E-cadherin, Vimentin), microvessel density (MVD) by CD34 in HCC.

Methods: One hundred ninety patients with HCC underwent radical resection were enrolled. Preoperative serum CEA (cut-off value: 5.0 ng/ml) level was measured and divided into two groups (high and normal), and evaluated the relationships to clinicopathological factors, CEACAM1 expression, EMT-related factors, and MVD.

Results: In disease-free survival (DFS) rate was significantly worse in the CEA high group (5 DFS rate: normal vs high/66.0 vs 46.3 % $p < 0.01$). In multivariate analysis, high CEA levels (HR2.42, 95 % CI 1.31–4.30 $p < 0.01$), multiple tumors (HR2.14, 95 % CI 1.27–3.55 $p < 0.01$) were identified as independent recurrence prognostic factors. Furthermore we examined the correlation with CEA levels and EMT-related factor, CEACAM1, MVD. Vimentin was higher, and E-cadherin was lower in the high CEA group ($p < 0.01$). Also CEACAM1 expression was higher, further CEACAM1 expression correlated with the EMT markers ($p < 0.05$). In addition, MVD was higher in the high CEA group ($p < 0.01$).

Conclusion: Serum CEA levels is associated with EMT, tumor angiogenesis, the promising prognostic marker after hepatectomy.

P-0592

ELK4, SIRT7 and H3K18ac expressions in hepatocellular carcinoma with prognostic implication

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Background: ETS-like transcription factor 4(ELK4/Sap-1) belongs to the ternary complex factor (TCF) subfamily of ETS domain transcription factors. Compared to the other TCF subfamily members, the mechanisms of regulation of ELK4 are not much known. ELK4 is known to form complex with NAD-dependent deacetylase sirtuin-7 (SIRT7). Recruited SIRT7 induces deacetylation of H3K18ac and suppresses transcription of many genes including tumor suppressor loci. We investigated this ELK4-SIRT7-H3K18ac pathway in hepatocellular carcinoma tissues.

Materials and methods: 278 of hepatocellular carcinoma (HCC) patients were enrolled in the study. Immunohistochemical expression of ELK4, SIRT7, and H3K18ac was scored and analyzed with clinicopathologic factors.

Results: High expression of ELK4, SIRT7, and H3K18ac was observed in 66 (24 %), 51 (19 %) and 143 (55 %), respectively. In univariate survival analyses, high expression of ELK4 was associated with increased overall survival ($P = 0.021$), and high expressions of SIRT7 and H3K18ac were correlated with poor survival ($P = 0.034$ and $P = 0.001$). In multivariate survival analyses, ELK4 and H3K18ac were also independent prognostic factors ($P = 0.015$ and $P = 0.001$).

Discussion: High expression of SIRT7 showed worse prognosis. But, high ELK4 group showed better survival outcome and high H3K18ac group showed worse survival outcome. ELK4-SIRT7-H3K18ac pathway may be interrupted by more influential factors such as MAPK signaling pathway, oncogene-induced senescence surveillance, global histone modification, compensation and tumor doubling rate. ELK4 and H3K18ac are independent prognostic factors of HCC.

P-0593

Lymphotoxin- β regulated by NF- κ B is a biomarker of poor prognosis in hepatocellular carcinoma

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The Lymphotoxin- β (LT β) is a major pro-inflammatory cytokine that belongs to the TNF superfamily and mediates local inflammatory responses as a membrane-bound cytokine and considered to be important in the hepato-carcinogenesis. We previously reported that the CCCTC-binding factor (CTCF) mediated higher order chromatin conformation of TNF/LT locus is deeply involved in the regulation of LT β expression in human HCC, in terms of the global analysis with ChIP-chip and ChIP-seq. In this study, we attempted to reveal the molecular mechanism of LT β regulation and the clinical characteristics of the patients with HCC expressing LT β . Firstly, we found the constitutive active NF- κ B was deeply involved in LT β expression due to alteration of higher order chromatin conformation controlling

the interaction between LT β promoter and NF- κ B-responsive enhancer. Because we could quantify LT β in the exosomes extracted from patient serum, we described that high level of exosomal LT β reflected the high expression LT β in HCC tissues evaluated by immunohistochemistry in 24 patients. Moreover, patients with the high level of exosomal LT β exhibited significantly shorter survival time. Therefore, these results suggest that exosomal LT β might be a useful biomarker related to poor prognosis in HCC.

P-0594

The diagnostic accuracy for early stage hepatocellular carcinoma by combined use of AFP and PIVKA-II

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Background: Hepatocellular carcinoma (HCC) is one of the leading causes of death in Taiwan and the fifth most common cancer worldwide for many years. α -fetoprotein (AFP) and protein induced by vitamin K absence or antagonist-II (PIVKA-II) are currently used to screen HCC. However, for early stage HCC, the optimal cutoff values for diagnosis and the clinical factor affecting its accuracy remain controversial. Thus, the purpose of this study was to investigate clinical factor affecting the diagnostic accuracy for early stage HCC by combined use of AFP and PIVKA-II.

Method: A number of 482 patients with early stage HCC were retrospectively enrolled. Levels of AFP and PIVKA-II in serum were measured by chemiluminescence enzyme immunoassay (CLEIA). Risk factors of prognosis for overall survival were analyzed, such as characteristics of patients, tumor and treatments. For survival analysis we used the Kaplan–Meier technique, with a log-rank test to detect the differences in cumulative survival rates.

Result: The mean age among patients was 63.15 years (SD 11.39 years). 225 patients (47 %) were hepatitis B surface antigen infection and 167 (35 %) patient had hepatitis C infection. For the relationship between AFP and PIVKA-II levels, no correlation was found between serum AFP and PIVKA-II levels ($R^2 = 0.068$). The factor of PIVKA-II had influences on overall survival with statistical significance ($P = 0.001$) in early stage HCC patients with low levels of AFP.

Conclusion: This study revealed that PIVKA-II plays an important role even in early stage HCC patients with low levels of AFP.

P-0595

Value of AFP, AFP-L3 and DCP in early detection of hepatocellular carcinoma in Vietnamese patients

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Aim: To evaluate value of AFP, AFP-L3 and DCP in combination with imaging diagnostics in early detection of hepatocellular carcinoma (HCC).

Methods: Sera from patients infected hepatitis B or C virus or had abnormal ultrasound results were measured AFP, AFP-L3 and DCP

by μ TASWako i30 and performed abdominal Doppler ultrasound, CT scan or MRI with Primovist. The threshold value of AFP, AFP-L3, DCP supposed elevated indicators was >40 , >20 and >80 , respectively.

Results: Of 456 patients, 68 (14.91 %) patients had elevated AFP, 23 (5.04 %) had elevated AFP-L3, 48 (10.53 %) had elevated DCP and 102 (22.37 %) had at least one of the three elevated markers. In these 102 patients, 74 (72.55 %) were male and 28 (27.45 %) were female and the proportion of male patients having elevated AFP-L3 or/and DCP more than four times higher than the female ($p < 0.001$). 98 and 57 in 318 patients infected HBV or HCV got at least one elevated marker (30.82 %) and elevated AFP-L3 or/and DCP (17.92 %), respectively ($p < 0.01$). 69.70 % patients with at least one elevated had abnormal ultrasound results ($p < 0.001$). All 23 patients having abnormal CT scan and 15 in 16 patients having abnormal MRI results got elevated AFP-L3 or/and DCP ($p < 0.001$).

Conclusion: Sex and HBV or HCV infection supposed risk factors of HCC correlated with elevation of AFP, AFP-L3 and DCP. Measuring AFP, AFP-L3 and DCP combined with ultrasound was a cost-effectiveness and affordable tool in screening HCC patients. Especially, AFP-L3 and DCP markers could improve the effectiveness in early detection of HCC.

P-0596

Expression of SALL4 in hepatocellular carcinoma and its clinical significance

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Objective: To confirm the correlation between SALL4 expression and progression and prognosis of hepatocellular carcinoma and its potential as an independent prognostic factor for HCC. To clarify the relationship between SALL4 expression and cancer treatment response.

Methods: 10 cases of HCC treated by tumor resection or liver transplantation were selected. The medical records of these patients were retrospectively analyzed. The data including demographic data such as age, gender, basic disease, pathological data such as tumor size, tumor stage, fibrosis, and lymphocyte infiltration and clinical data were collected. The expression of SALL4 in tissues was evaluated by immunohistochemistry and detected by Western blot. The correlation between SALL4 expression and liver cancer progression and prognosis was evaluated by combining with the above clinical data.

Results: The SALL4 expression was positive in one case and the pathological features were that the SALL4 expression was detected in the nucleus and DAB color reaction was positive. We detected the SALL4 expression of the positive samples by Western blot. The results showed that SALL4 was highly expressed in HCC tissues compared to healthy controls and tumor-adjacent tissues. The clinical data of the patient indicated that the disease was malignant progression.

Conclusion: our results show that the expression of SALL4 is correlated with the malignant progression and prognosis of HCC, and it may become an independent prognostic factor for HCC. More cases are needed to confirm the results and to further determine the relevance of the sensitivity to chemotherapy.

P-0597

The impact of neutrophil/lymphocyte ratio for the recurrence of HCC after RFA

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Radio frequency ablation (RFA) is an established treatment for small hepatocellular carcinoma (HCC), and the control of recurrence is essential for long survival after RFA. Recently neutrophil-to-lymphocyte ratio (NLR) was suggested to be the prognostic marker of HCC. Therefore we evaluated predictive factors including NLR to be associated with the recurrence after curative RFA. A hundred sixty three patients who had been initially diagnosed with HCC and had been treated with RFA were enrolled. We retrospectively analyzed factors which are associated with the recurrence and survival after RFA. Hepatitis C virus (HCV) infection was most frequent causes of HCC (68.1 %), whereas hepatitis B virus (HBV) infection was found in 26 cases (16.0 %). Recurrence, almost intra-hepatic distant recurrence, was found in 101 cases (61.9 %) and median time for recurrence was 31 months. Recurrence and posttreatment NLR were independent prognostic factors related to survival, and male, HCV infection, serum DCP >40 AU/L and posttreatment NLR were associated with the recurrence. Pretreatment NLR showed no association with the recurrence, whereas posttreatment NLR showed the prognostic value. On the other hand, pretreatment NLR >2.5 was significantly associated with the recurrence in HBV-HCC patients (OR 3.439, $P = 0.037$) not but HCV-HCC (OR 1.430, $P = 0.17$). In conclusion, recurrence of HCC after RFA was strongly associated with survival. NLR is suggested to be a predictive marker of recurrence especially in HBV-HCC patients.

P-0598

Expression analysis of plasma apolipoprotein AI and AIV in hepatocellular carcinoma

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Aim: To find out differential expression of apolipoproteins- ApoA1 and ApoAIV in HCC and controls without HCC.

Methods: 50 Patients with HCC and 50 liver cirrhosis were included in the study. Proteins were depleted and precipitated followed by separation in SDS PAGE and ApoA1 and ApoAIV were confirmed by Western Blotting followed by densitometric protein semiquantitation estimation along with ELISA based protein quantification.

Results: Western blotting densitometry image analysis of the plasma samples following CDC protocol and the comparison between patients with and without HCC revealed differential expression of ApoA1 and ApoAIV. Levels of Apo-AIV were significantly higher in patients of liver cirrhosis without HCC than in patients with HCC (0.208 ± 0.07 and 0.119 ± 0.016 versus 0.119 ± 0.005 ; $P < 0.01$). Levels of Apo-A1 were significantly higher in patients with HCC than in controls without HCC (0.279 ± 0.003 vs 0.171 ± 0.034 and 0.199 ± 0.014 ; $P < 0.01$). Elisa result showed significant increased ApoA1 expression in HCC group ($P < 0.01$).

Conclusion: Apolipoprotein A1 is highly expressed in HCC in comparison to Cirrhosis and may be used as future diagnostic tool in

addition and associated with other conventional biomarkers for HCC after further analysis higher number of population.

P-0599

Diagnostic performance for small hepatocellular carcinoma measuring less than 2 cm

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Background: Recent guidelines recommend ultrasound follow-up for nodules (<1 cm) discovered via ultrasonography surveillance in cirrhosis patients. There is no specified recall policy of nodule less than 1 cm. We evaluated CT and MRI efficacy in HCC diagnosis in nodules lesser than 1 cm and 1–2 cm.

Methods: We retrospectively analyzed 130 US-detected liver nodules in lesser than 2 cm, which were confirmed histologically. The two strategies were single imaging scan only, and two imaging scans. 1 Single image scan only (CT or MRI) and (2) two imaging scans; combining 2 contrast enhanced imaging scans.

Results: Histologically, HCC was confirmed in 110 of 130 (84.6 %) nodules; 29 of 36 (80.5 %) nodules (<1 cm), and 81 of 94 (86.2 %) nodules (1–2 cm) were HCCs. Imaging characterized 24 of 29 HCCs (<1 cm) with the typical HCC vasculature (82.7 %; single image positive: 4; two image positive: 20). In nodules (<1 cm), sensitivity, positive predictive value (PPV), and diagnostic accuracy were 80.0, 66.6, and 66.6 % for single, and 83.3, 95.2, and 81.4 %, for two images, respectively. Of 81 HCC nodules measuring 1–2 cm, 79 possessed the typical HCC vasculature (97.5 %; single image: 24; two images: 55). In (1–2 cm) HCC nodules, sensitivity, PPV, and diagnostic accuracy were 96.0, 92.3, and 90.3 % for single, and 98.2, 94.8, and 93.6 %, for two images.

Conclusions: In HCCs (<1 cm), our study suggests that two imaging modalities provided limited diagnostic efficacy; invasive biopsy may be needed. In HCCs (1–2 cm), single or two imaging modalities produced similar diagnostic efficacy.

P-0600

SPIO-enhanced MRI is useful in predicting hypervascularization of hypointense hypovascular nodules

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Aims: To examine whether superparamagnetic iron oxide (SPIO)-enhanced MRI can be used to assess the malignant potential of hepatic hypovascular nodules showing hypointensity during the hepatobiliary phase on Gd-EOB-DTPA-enhanced MRI.

Methods: The study included 42 patients with chronic liver disease who had small hypovascular nodules showing hypointensity during the hepatobiliary phase on Gd-EOB-DTPA-enhanced MRI. SPIO-enhanced MRI analyzed whether the signal intensity of each nodule

was high. Nodules were prospectively followed-up until hypervascularization by periodic Gd-EOB-DTPA-enhanced MRI. Initial MRI findings and clinical variables were used to analyze potential predictive factors for hypervascularization.

Results: The study enrolled 77 nodules, of which 19 (25 %) showed hypervascularization during the observation period. The cumulative rates for hypervascularization were 11 % at 1 year and 22 % at 2 years. Hyperintensity was observed in 12 nodules (16 %) on SPIO-enhanced MRI; among these, 7 (58 %) showed hypervascularization, on the other hand, 12 (18 %) of the remaining 65 nodules without hyperintensity showed hypervascularization ($P = 0.07$). A Cox model revealed that independent predictors of hypervascularization included hyperintense nodules on SPIO-enhanced MRI ($P < 0.001$). The cumulative rates for hypervascularization in hyperintense nodules were 52 % at 1 year as per SPIO-enhanced MRI, whereas these rates were 3 % for nonhyperintense nodules.

P-0601

Usefulness of angio CT for the diagnosis and treatment of early hepatocellular carcinoma

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Aim: Early hepatocellular carcinoma (eHCC) is defined as well-differentiated HCC with an obscure tumor margin in the classification of the Liver Cancer Study Group of Japan. In this study, the value of Angio CT for the diagnosis and treatment of eHCC was examined.

Method: The imaging features of Angio CT, dynamic CT, EOB-DTPA MRI and contrast enhancement ultrasonography (CEUS) of 52 eHCC nodules in 42 cases were investigated. The diagnosis of all the nodules was definite by histological examination.

Results: 50 of 52 eHCC nodules showed hypointensity on hepatobiliary-phase (HP) of EOB-MRI. And 25 of 50 eHCC nodules with hypointensity on HP of EOB-MRI also revealed hypoechoic pattern in postvascular phase of CEUS. In remaining 25 nodules without hypoechoic pattern in postvascular phase of CEUS, 15 of 20 nodules less than 20 mm in tumor diameter showed low density areas in CTAP. 12 of these 15 nodules were treated with RFA. But untreated 3 nodules became hyper vascular nodules within 2 years.

Conclusion: In the Japanese diagnostic guideline of hypo vascular hepatic nodule, observation is recommended in the nodules less than 20 mm in tumor diameter that reveal hypointensity on only HP of EOB-MRI without hypoechoic pattern in postvascular phase of CEUS. But this study showed that in eHCC, 75 % of all nodules less than 20 mm in tumor diameter showed the reduction of portal flow and predicted to be hyper vascular nodules in the near future. It may be possible to select these nodules by performing Angio CT in the diagnosis of eHCC.

P-0602

A strategy to diagnose hepatocellular carcinoma by EOB-MRI

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Aim: Recently, Gadolinium ethoxybenzyl diethylenetriamine pentaacetic acid enhanced MRI (EOB-MRI) is frequently used for the diagnosis of small HCC. However, it is sometimes difficult to distinguish dysplastic nodules from hypovascular HCC without biopsy. The aim of this study was to elucidate a way to know malignant potential of hypovascular hepatic nodules with EOB-MRI.

Methods: The subjects were 182 hypovascular nodules (132 patients) that were detected as hypointense liver lesions at the hepatic arterial phase of EOB-MRI during the surveillance of HCC. All of them were histologically diagnosed by tumor biopsy. We analyzed the correlation between patients' characteristics including the finding of the imaging data and the histological findings. We also tried to find out a diagnostic strategy by data mining.

Results: Among 182 nodules, 127 were diagnosed as HCC and 55 as non-HCC. In univariate analysis, male, age, tumor size, viral infection, hyper-intensity at DWI, hypo-intensity at delayed phase and hepatobiliary phase (HBP) were significant risk factors for HCC. Multivariate analysis with the factors revealed that tumor size ($HR = 4.06$, $P = 0.002$) was only significant risk factors for HCC. The data mining with EOB-MRI imaging showed that the first meaningful image was DWI. The findings of hepatobiliary phase, delayed phase, and prior history of HCC were selected as other significant factors and the possibility of HCC were stratified between 18.2 and 97.1 %.

Conclusion: Possibility of HCC could be predicted with the findings of EOB-MRI.

P-0603

Specific phosphatidylcholine species are associated with liver tumors detected by 18F-choline PET/CT

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PET/CT imaging with 18F-choline is a potential diagnostic modality for hepatocellular carcinoma (HCC) that is based on depicting the initiating steps of tissue phosphatidylcholine (PtC) synthesis. To relate the diagnostic performance of 18F-choline PET/CT with the phospholipid composition of liver cancers, we profiled the liver tumors of 23 patients who underwent PET/CT prior to hepatectomy. Orbitrap liquid chromatography mass spectrometry was used to quantify over 200 molecular species of PtC in 16 fractions, with principal component (PC) analysis reducing the results into 3 factors accounting for 75 % of total profile variation. PtC species containing poly-unsaturated fatty acids were the only significant contributors to the first PC factor, which also clearly separated intrahepatic cholangiocarcinoma from HCC tumors. PtC species containing saturated fatty acids were the only significant contributors to the second PC factor, which was the only factor that strongly correlated with tumor uptake on PET ($r = 0.63$, $p = 0.0012$). The overall tumor detection rate of PET was 94 % based on finding any change in hepatic 18F-choline uptake. The detection rate for HCC was 84 % if a positive PET finding was defined by only an increase in tumor 18F-choline uptake, in which case HCC tumors failing detection were associated with lower levels of highly-saturated PtC species. In conclusion,

phosphatidylcholine composition varies in primary liver cancer and is associated with tumor detectability on 18F-choline PET/CT. In addition to having potential diagnostic value, imaging with 18F-choline may help in monitoring treatments affecting CDP-choline pathway mediated PtC synthesis in HCC.

P-0604

Importance of tumor size as a prognostic factor after liver resection for solitary HCC

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Background: Presently, the impact of tumors size as a prognostic factor after curative liver resection (LR) for solitary hepatocellular carcinoma (HCC) remains controversial. This study was performed to determine the prognostic factors of patients undergoing LR for solitary HCC with special emphasis on the importance of tumor size.

Methods: Between 2000 and 2013, 560 patients underwent curative LR for solitary primary HCC which met the study criteria.

Results: One-hundred and seventy-eight patients underwent major hepatectomies and the overall in-hospital mortality was 2.0 %. There were 282 patients (50.4 %) with liver cirrhosis. The 5-year overall survival was 64 % and recurrence-free survival was 50 %, respectively. Multivariate analyses demonstrated that cirrhosis, microvascular invasion and size were independent predictors of RFS and cirrhosis, microvascular invasion and age were independent prognostic factors of OS. Subset analysis demonstrated that tumor size was a prognostic factor for solitary HCC with microvascular invasion (AJCC T2) but not solitary HCC without microvascular invasion (AJCC T1).

Conclusions: Size, microvascular invasion and cirrhosis are independent prognostic factors of RFS for solitary HCC after LR. Tumor size is an important prognostic factor in T2 but not T1 solitary tumors. These findings suggest that the current AJCC TNM staging system may need to be revised.

P-0605

The necessity of computed tomography to hepatic lesions detected on private clinic sonography

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Background: We meet more patients with hepatic lesions than past as more private clinic do abdominal sonography. As a result, the prescription of computed tomography has been markedly increased. We aimed to evaluate that computed tomography should be needed in patients who was referred for hepatic lesions from private clinic.

Methods: We enrolled patients from Jan. 2010 to Apr. 2014 who were referred for further evaluation of intrahepatic lesion detected on private clinic abdominal sonography. The diagnosis on sonography, confirmed diagnosis on computed tomography, sensitivity of them was analyzed.

Results: Total 116 patients were enrolled. 126 hepatic lesions were detected on private clinic sonography and 163 hepatic lesions were detected on computed tomography. Most common diagnosis was hemangioma (22.2 %), followed by cyst (12.7 %), HCC (10.3 %), RN/DN (4 %), focal fat sparing (3.2 %), undetermined (44.4 %). They were confirmed as hemangioma (26.7 %), cyst (24.6 %), HCC (8 %), focal fat sparing (4.8 %), RN/DN (3.2 %) on computed tomography. 20 (15.9 %) hepatic lesions on sonography were not shown on computed tomography. Sensitivity for hemangioma, cyst, HCC, RN/DN was 82.1, 68.8, 53.8, 60. 53 lesions (98.1 %) of 54 benign lesions on sonography were confirmed as benign lesions on computed tomography. Only 1 lesion (1.9 %) suspected as RN/DN in patient with LC was confirmed as HCC.

Conclusion: Although sensitivity of hepatic lesions on private clinic sonography is 53.8–82.1 %, specificity for malignant lesions is very high. We should consider carefully computed tomography for benign hepatic lesions suspected on private clinic sonography to avoid abuse.

P-0606

Preoperative liver functional volumetry performed by 3D-99mTc-GSA scintigraphy/vascular fused images

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Purpose: The present study was designed to evaluate the feasibility of preoperative liver functional volumetry performed by 3D-technetium-99m-diethylenetriaminepentaacetic acid-galactosyl-human serum albumin (99mTc-GSA) scintigraphy/vascular fusion imaging using SYNAPSE VINCENT and to examine the discrepancy between conventional and functional volumetry.

Methods: The study group comprised 17 patients who underwent preoperative 3-dimensional (3D)-99mTc-GSA scintigraphy/vascular fusion imaging using SYNAPSE VINCENT software before hepatectomy between July 2014 and August 2015. The diagnosis was hepatocellular carcinoma (n = 5), metastatic liver tumor (n = 10), or intrahepatic cholangiocarcinoma (n = 2). Right hepatectomy was performed in 3 patients, left hepatectomy in 3 patients, right posterior sectionectomy in 3 patients, left medial sectionectomy in 1 patient, segmentectomy in 2 patients, and partial hepatectomy in 5 patients. 99mTc-GSA scintigraphy and computed tomography (CT) were performed to construct 3D-99mTc-GSA scintigraphy/vascular fused images. The conventional volume ratio of the planned resection region without tumor (%CT), and the functional volume ratio of the planned resection region without tumor (%GSA) were calculated. The discrepancy ratio was calculated as follows: discrepancy ratio = $100 - \%GSA/\%CT \times 100$ (%).

Results: The %GSA (19.8 ± 16.3 %) was significantly lower than the %CT (24.0 ± 18.0 %) ($p < 0.05$). In all except 2 patients, the %GSA was lower than the %CT. The discrepancy ratio ranged from -4 % to 75 % (median 19.5 %).

Conclusions: 3D-99mTc-GSA scintigraphy/vascular fused images constructed using SYNAPSE VINCENT were useful for noninvasively performing functional liver volumetry in patients scheduled to undergo various patterns of hepatectomy. In planned resection regions without tumor, the functional volume ratio was about 20 % lower than the conventional volume ratio.

P-0607

Lack of contrast washout in delayed phases in hepatocellular carcinoma with portal vein occlusion**Ye Eun Kwak^{1,2}, Caroline Loeser^{1,2}**¹Bridgeport Hospital, Department of Medicine, Yale University School of Medicine, Bridgeport, USA; ²Bridgeport Hospital, Section of Gastroenterology, Department of Medicine, Yale University School of Medicine, Bridgeport, USA**Background:** Hepatocellular carcinoma (HCC) can be diagnosed radiologically with arterial enhancement and delayed washout pattern in contrast enhanced images by CT or MRI. Repeat images with different modalities in many cases are inconclusive and invasive liver biopsy is required.**Aim:** HCC can lack contrast washout in delayed phases of MRI when there is extensive occlusion of portal vein by thrombosis.**Methods:** We present a 61 year old male with decompensated liver cirrhosis with portal vein thrombosis who was diagnosed with HCC by liver biopsy after multiple inconclusive imaging studies.**Results:** 61 year old male with a history of alcohol abuse and hepatitis C infection presented with severe abdominal distension. Ultrasound revealed liver cirrhosis, portal vein thrombosis, splenomegaly and large ascites. CT with intravenous contrast revealed liver cirrhosis with several small low-attenuation foci and portal vein thrombosis. MRI hepatoma protocol revealed liver cirrhosis with extensive occlusion of portal vein and up to 3 cm heterogeneous arterial enhancement in right and left lobes lacking washout in delayed images. Repeat MRI was inconclusive. Liver biopsy revealed poorly differentiated HCC diffusely infiltrating cirrhotic liver parenchyma.**Conclusions:** HCC is supplied by hepatic artery whereas liver parenchyma is supplied by both hepatic artery and portal vein. Occlusion of portal vein by thrombosis alters blood supply to the liver resulting in lack of delayed washout which is also observed in intrahepatic cholangiocarcinoma. We suggest absence of delayed washout should not exclude HCC or lead to conclusion of ICC when significant portal vein occlusion is present.

P-0608

Comparison of 'low MI THI' mode and CHI mode in contrast-enhanced ultrasonography of liver tumors**Takeshi Nihei¹, Kojiro Asano¹, Nagoya Kanogawa¹, Yohtaro Iino¹, Ken Ohkawara¹, Naoaki Konno¹, Akari Munakata¹, Kohji Watanabe¹, Hiroshi Kashimura¹, Takehiko Mimura², Keiko Andoh², Masahiko Shimada²**¹Department of gastroenterology, Mito Saiseikai General Hospital, Mito, Ibaraki, Japan; ²Division of radiological technology, Mito Saiseikai General Hospital, Mito, Japan**Background:** Contrast-enhanced sonography is indispensable method for diagnosis and treatment for liver tumors. Usually we use CHI mode, but this mode has several weak points, such as poor visibility in deep area and inferior real-time property due to fewer frame rate.**Methods:** Examinations were performed with Aplio XG and XV (Toshiba medical inc.). We use following 2 methods for taking images in contrast-enhanced sonography. One is CHI mode, MI ranged from 0.21 to 0.37, dynamic range 40, frame rate ranged from 10 to 12. Another is 'low-MI THI' mode, MI ranged from 0.4 to 0.5, dynamic range 40, frame rate ranged from 12 to 19. 20 liver tumors,

which consist of 13 Hepatocellular carcinoma, 7 benign nodules, were examined. In vascular phase of contrast enhancement, following 5 factors, description of tumor vessel, visibility of enhancement, contrast of enhancement, visibility of background liver and diagnostic ability, were estimated by 2 gastroenterologists and one sonographer with more than 10 years experience of liver imaging.

Results: 'Low-MI THI' mode is superior to CHI mode in description of tumor vessel, visibility of enhancement, visibility of background liver and diagnostic ability, but only in contrast of enhancement. This superiority was seen regardless of the depth of the lesion.**Conclusions:** 'Low-MI THI' mode might almost be superior to CHI mode in description of liver tumors in vascular phase of contrast-enhanced sonography.

P-0609

Comparison of the latest fusion technology**Hironori Tanaka, Tamaki Takata, Kazumi Kawabata, Yuma Inoue, Yoshihito Inoue, Takashi Uchihashi, Hayato Miyamoto, Yuko Hanasaki, Koichi Ikee, Yasuyoshi Yamasaki, Kosuke Tamura, Hiroshi Yunokizaki, Tyoryo Ri, Junichi Miyazaki, Takashi Abe**

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Background: Ten year or more passed after real-time virtual sonography (RVS), which is the first fusion technology, becomes available in 2004. Now, there are already five US models equipped fusion technology, and that technology is now important for radiofrequency ablation therapy of liver cancer. Because these techniques have progressed year by year, comparison of those technologies is not available yet. Therefore, we compared those characteristics among the 5 latest US models equipped fusion technology.**Method:** From January 2015 to July 2015, we used five US models equipped fusion technology (Hitachi Aloka Ascendus, GE LOGIQ E9, Toshiba Aplio 500, Philips EPIQ 7G, Siemens S3000), and compared those all characteristics.**Result:** (1) Auto registration function was available in three models (LOGIQ E9, active patient tracker; EPIQ 7G, vessel base and surface base; S3000; Auto-alignment Methods). (2) US volume data was available in three models (Ascendus, LOGIQ E9, EPIQ 7G). (3) MRI was available in 4 models except S3000. (4) Ascendus could show 3 other time phase at the same time. (5) 3D body marker was available in three models (Ascendus, Aplio 500, EPIQ 7G). (6) Immediate image change was available in Ascendus and LOGIQE9. (7) Immediate change between B-mode and contrast enhanced US was available 4 models except EPIQ 7G. (8) Needle navigation system was available in three models (Aplio 500, LOGIQ E9, EPIQ 7G).**Conclusion:** The fusion technology continues progressing. We expect the further progress of the fusion technology.

P-0610

Usefulness of a three-dimensional simulator system for multipolar ablation**Masashi Hirooka, Yohei Koizumi, Yoshiko Nakamura, Yusuke Imai, Takao Watanabe, Osamu Yoshida, Yoshio Tokumoto, Masanori Abe, Yoichi Hiasa**

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Background: Although multipolar radiofrequency ablation is expected to produce a large ablated area and reduce intrahepatic recurrence of hepatocellular carcinoma, performance requires considerable skill. This study evaluated the utility of a new simulator system for multipolar radiofrequency ablation. To understand the positioning of multipolar electrodes on three-dimensional images, we developed a new technology by expanding real-time virtual ultrasonography.

Methods: We performed experimental punctures 21 times in phantoms. In phantom and study, directions and positions of electrodes were confirmed on computed tomography, and the accuracy and utility of the simulator system were evaluated by measuring angles and intersections for each electrode.

Results: In cases of puncture with 2 electrodes, correlations between angles on each imaging modality were also strong [ultrasound (US) vs. simulator: $r = 0.991$, $p < 0.001$, simulator vs. computed tomography (CT): $r = 0.991$, $p < 0.001$, US vs. CT: $r = 0.999$, $p < 0.001$]. Correlations between distances in each imaging modality were also strong (US vs. simulator: $r = 0.993$, $p < 0.001$; simulator vs. CT: $r = 0.994$, $p < 0.001$; US vs. CT: $r = 0.994$, $p < 0.001$). In cases with 3 electrodes, distances between each electrode correlated strongly (yellow-labeled vs. red-labeled: $r = 0.980$, $p < 0.001$; red-labeled vs. blue-labeled: $r = 0.953$, $p < 0.001$; yellow-labeled vs. blue-labeled: $r = 0.953$, $p < 0.001$).

Conclusions: The new simulator system appears to provide accurate locations of electrodes. This simulator system could allow multipolar radiofrequency ablation to be performed more effectively and comfortably.

P-0611

US–US fusion imaging in radiofrequency ablation therapy for liver cancer

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Background: It is often difficult to assess the ablation margin of radiofrequency ablation (RFA) therapy on ultrasonography (US). In contrast, US–US fusion imaging can display the synchronous images of before/after RFA side-by-side according to the US probe action, and ablation margin of RFA might be evaluated three-dimensionally immediately after RFA. AIM. This study investigated the effectiveness of US–US fusion imaging in RFA for liver cancer.

Materials and methods: Between October 2014 and October 2015, 54 patients with 74 liver cancer (hepatocellular carcinoma $n = 67$, metastatic liver cancer $n = 7$) were enrolled. Before ablation, three dimensional volume data of US were obtained by sweep scanning, and we traced the edge of liver cancer for coloring the entire tumor on the data. After ablation, a hyperechoic area was seen on B-mode US. US–US fusion imaging showed the synchronous images of before/after RFA side-by-side after images registration. Moreover, an overlay of US images before/after RFA could reveal an ablation margin three-dimensionally by showing the colored tumor inside hyperechoic area.

Results: The maximal diameters of all tumors ranged from 0.8 to 5.0 cm (mean 1.9 cm) on US. Complete tumor necrosis was achieved by a single session of RF ablation in all patients. We did not encounter local tumor progression and severe complication during the observation period.

Conclusion: US–US fusion imaging in RFA is an efficient approach for liver cancer, and have potential to cancel early CT/MRI assessment of treatment response if ablation margin could be accurately evaluated by US–US fusion imaging immediately after RFA.

P-0612

Which is useful for liver carcinogenesis prediction needle biopsy or liver stiffness measurement?

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Aims: We can use new ultrasonographic methods for non-invasive diagnosis of liver fibrosis. We made it clear whether the Virtual Touch Quantification (VTQ) is an alternative to needle biopsy.

Methods: (1) For 1619 patients (HBV272/HCV827/BC11/NBNC509) were evaluated liver fibrosis stages by needle biopsy and VTQ. We examined the liver cirrhosis diagnostic performance of VTQ in ROC analysis. (2) Among them we followed up 1434 patients who did not had past history of HCC and compared the carcinogenic evaluation capacity of the needle biopsy and VTQ.

Result (1) AUROC of dividing the F0-3 and F4 were HCV/HBV/AIH/PBC/NASH 0.861/0.879/0.873/0.948/0.893 and cut-off value were 1.63/1.40/1.66/1.63/1.64 m/s. HBV was softer than other etiologies significantly. (2) Their fibrosis stages; F0/1/2/3/4 were 50/629/279/296/180 cases and their VTQ value were 1.09/1.17/1.35/1.63/2.21 m/s. 36 patients developed HCC (F1/2/3/4 5/4/9/18 cases). The univariate analysis there were significant difference in age, sex, fibrosis stages, activity stages, platelet counts, VTQ and fasting plasma glucose. Cut-off value of VTQ for carcinogenesis prediction was 1.35 m/s in ROC analysis. Then we were stratified VTQ; less than 1.35/1.35–1.60/1.60–2.00/more than 2.00 and 5-year cumulative carcinogenic rate was 3.5 % in < 1.60 m/s, 8.1 % in 1.60–2.00 m/s and 23.2 % in 2.00-m/s by the Kaplan–Meier. By same way 5-year cumulative carcinogenic rate was 2.8 % in less than F2, 6.3 % in F3 and 19.3 % in F4 stage.

Conclusion: VTQ has a high capacity for diagnosis of liver cirrhosis. Cumulative liver carcinogenesis rate increases significantly in proportion to the F stages and VTQ. So we think that the evaluation of cirrhosis diagnosis and cancer risk is enough non-invasive inspection.

P-0613

Kupffer images of contrast ultrasound and recurrence after hepatectomy for hepatocellular carcinoma

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Aims: The present study aimed to evaluate pathologic features of hepatocellular carcinoma (HCC) appearing an irregular defect on Kupffer phase of contrast-enhanced ultrasonography (CEUS) with perfluorobutane microbubbles, and to elucidate the association between Kupffer-phase images and metastatic recurrences after hepatectomy.

Methods: A total of 73 patients with solitary HCC under 5 cm in diameter who underwent CEUS before hepatectomy were analyzed for the evaluation of pathologic features. Excluding 2 patients who died within 3 months after hepatectomy, 71 patients were used for the analysis of the factors contributing metastatic recurrence after hepatectomy. Intrahepatic metastatic recurrence was defined as >3 intrahepatic recurrences. The irregular type included HCC with an irregular or unclear contour on conventional B-mode or with an irregular defect on the Kupffer-phase images.

Results: The accuracy of Kupffer-phase images for predicting the non-single-nodular type were significantly higher than that of conventional B-mode (74 vs. 92 %, $p < 0.01$). Serum des-gamma-carboxyprothrombin levels and the percentages of microscopic portal invasion and intrahepatic metastasis in the irregular group were significantly higher than those in the non-irregular group. The cumulative 5-year metastatic recurrence rates of the irregular and non-irregular groups were 56 and 43 % ($P = 0.028$), respectively. Multivariate analyses indicated that des-gamma-carboxyprothrombin levels and Kupffer-phase images were significant factors related to metastatic recurrence.

Conclusions: Kupffer-phase images can more accurately predict the gross type than conventional B-mode. HCCs with an irregular defect on Kupffer-phase of CEUS are characterized by more frequent microscopic portal invasion and intrahepatic metastasis, and are significantly associated with metastatic recurrences after hepatectomy.

P-0614

Detection of small liver tumors using intraoperative real-time virtual sonography

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Background: In spite of the routine use of intraoperative ultrasonography (IOUS), we sometimes have trouble in finding small tumors. Recently, we introduced real-time virtual sonography (RVS), an innovative navigation technology that synchronizes an ultrasonography with preoperative imaging. We experienced two cases in which this navigation system was effective in detecting small liver tumors, which were difficult to detect using only IOUS.

Patient 1: A 67-year-old male underwent hepatic resection for hepatocellular carcinoma, which was 32 mm in diameter located in segment eight. Also there was another nodule which had not been confirmed to carcinoma, the size was eight mm in diameter, located in segment four. Intraoperatively, segment four nodule was identical as hyper echoic nodule, but it was indefinite that this nodule was identical as that detected in preoperative imaging. Intraoperative RVS was performed and made sure the nodule detected by IOUS was same as the nodule detected by preoperative imaging. Contrast enhanced IOUS revealed this nodule was benign tumor, so the only segment eight tumor was removed.

Patient 2: A 76-year-old female was diagnosed with rectal cancer with having synchronous liver metastasis located in segment five, the size was seven mm in diameter. She had numerous cysts in the liver

and made it difficult to detect this small tumor by ultrasonography. Intraoperatively, RVS helped surgeon to find tumor fast and easily, then tumor could be removed.

Conclusions: RVS is a novel navigation system and helpful to detect small tumors that are difficult to find using conventional intraoperative inspection.

P-0615

A risk score for predicting future occurrence of hepatocellular carcinoma in cirrhotic patients

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Background: Liver cirrhotic patients suffer from significantly high risks of hepatocellular carcinoma (HCC). Intermediate/late-stage HCCs are still commonly encountered despite regular screening. To enable early diagnosis, a simple score was developed to identify cirrhotic patients carrying the highest risk of HCC.

Methods: We recruited (A) 161 cirrhotic patients who had no prior HCC, and (B) 32 cirrhotic patients who had prior early-stage HCC (BCLC stage A) but achieved complete remission after therapy. Additionally, 300 cirrhotic patients were prospectively recruited, together with the originally recruited 161 cirrhotic patients (Total $N = 461$), for prospective validation study.

Results: A predictive score (R) was derived from univariate-multivariate analysis of 35 biochemistry and hemogram parameters. R was calculated by a logistic transformation of t, which was computed by the sugar/insulin ratio (SIR), HDL level (HDL), platelet count (PLT) and lymphocyte/neutrophil ratio (LNR): $t = -0.005 \text{ PLT} - 0.029 \text{ HDL} - 0.376 \log_{10}(\text{SIR}) + 0.854 \log_{10}(\text{LNR}) - 0.015 \text{ PLT} \times \text{LNR} + 0.062 \text{ SIR} \times \text{LNR} + 4.253$. Based on R, patients were stratified into four risk groups: high-risk ($0.65 < R$), intermediate-risk ($0.5 < R < 0.65$), minor-risk ($0.275 < R < 0.5$) and background-risk ($R < 0.275$). After follow-up for 2 years, stratified patient groups showed distinct Kaplan–Meier curves of cumulative HCC incidences (Log-rank $P < 0.001$), where the high-risk group showed the highest cumulative incidence of HCC in the prospective validation cohort.

Conclusions: A risk score was developed and validated for predicting HCC occurrence in liver cirrhotic patients. This score comprised only metabolic and immunological factors, suggesting their involvements in liver oncogenesis.

P-0616

Integration of albumin-bilirubin (ALBI) score into CLIP staging for hepatocellular carcinoma

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Background and aims: The albumin-bilirubin (ALBI) grade is a recently reported, simpler, more objective and evidence-based alternative to the Child-Pugh (CP) score for hepatocellular carcinoma

(HCC). We aimed to study whether ALBI grade could substitute for CP score in the Cancer of the Liver Italian Program (CLIP) for HCC. **Methods:** A cohort of 1731 Chinese patients with HCC treated in our institution was accrued to compare the prognostic performance of the CP-based and ALBI-based CLIP system, in terms of homogeneity, discriminatory ability and monotonicity of gradients which were numerically reflected by homogeneity likelihood and linear trend Chi-squares, c-indices, respectively.

Results: ALBI grade performed as well as CP score when integrated into the CLIP staging system in predicting clinical outcome (homogeneity likelihood Chi-square: 813.599 vs. 766.755; linear trend Chi-square: 358.981 vs. 312.629; c-index: 0.745 vs. 0.742). CP-based and ALBI-based BCLC systems were highly concordant with weighted κ value of 0.852. 608 (35.1 %) patients were upstaged by ALBI, whereas 3 (0.2 %) patients were downstaged by ALBI. All restaged patients showed significantly different clinical outcomes compared to their original stage classification.

Conclusions: The overall prognostic performance of ALBI-based and CP-based CLIP system was highly comparable.

P-0617

Prognostic scoring system for radiofrequency ablation: usefulness of ALBI-grade

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Background/aim: Recently, albumin-bilirubin (ALBI)-grade, which is proposed as a new grade for hepatic function, has been reported. We evaluated the efficacy of ALBI-grade and Child-Pugh classification in grading the patients by hepatic function and for predicting prognosis of hepatocellular carcinoma (HCC) who treated with radiofrequency ablation (RFA), retrospectively.

Method: From 2000 to 2014, 743 patients with naive HCC, who were treated with RFA, were enrolled. We made ALBI-T score which was consisted of ALBI grade and TNM stage of Liver Cancer Study Group (LCSGJ) like as Japan Integrated Staging (JIS) score. We compared JIS and ALBI-T score, retrospectively.

Results: Average age was 69.4 ± 8.9 years old. Average diameter and number of tumor were 2.1 ± 0.8 and 1.4 ± 0.8 cm (male:female = 501:242, Child-Pugh A:B:C = 568:171:4, ALBI grade 1, 2, 3 = 242:471:29, TNM of LCSGJ I:II:III:IV = 304:323:114:2) The number of JIS score 0, 1, 2, 3 and ALBI-T score 2,3,4,5,6 were 246, 294, 167, 36 and 103, 295, 247, 94, 4, respectively. The median survival time of JIS and ALBI-T scores were 87.4, 71.8, 52.2, 34.8 and 121.4, 78.4, 59.1, 41.3, 22.7 months, respectively.

Conclusion: ALBI-grade was thought to be better classification to distinguish patients with better hepatic function than Child-Pugh A, and ALBI-T score was thought to have good predicting value for prognosis of patients with HCC treated with RFA.

P-0618

SII index predicts outcome in HCC patients treated with sorafenib

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Background and Aims: We evaluated a systemic immune-inflammation index (SII) based on lymphocyte, neutrophil, and platelet counts and explored its prognostic value in patients with advanced hepatocellular carcinoma treated with sorafenib. Neutrophils can promote secretion of circulating growth factors such as VEGF and proteases. Lymphocytes play a crucial role in tumor defense by inducing cytotoxic cell death and inhibiting tumor cell proliferation and migration, thereby dictating the host immune response to malignancy.

Methods: 97 patients with advanced hepatocellular carcinoma (HCC) receiving sorafenib were available for our analysis. Lymphocyte, neutrophil and platelet was measured before beginning of treatment. Prediction accuracy was evaluated with area under the receiver operating characteristic curve (AUC).

Results: An optimal cutoff point for the SII of 360 stratified the patients with HCC into high (greater 360) and low SII (lower 360) groups in the training cohort. Univariate and multivariate analyses revealed the SII was an independent predictor for overall survival and relapse-free survival, and prognostic for patients with advanced HCC treated with sorafenib. Patients with SII lower 360 had better outcome than those patients with SII greater 360; median PFS 3.9 months (95 % CI 2.8–6.2) vs 2.6 months (95 % CI 1.8–3.3) ($p = 0.026$) and median OS 13.9 months (95 % CI 5.7–22.8) vs 5.6 months (95 % CI 3.2–10.4) ($P = 0.024$).

Conclusions: The SII was a powerful prognostic indicator of poor outcome in patients with advanced HCC treated with sorafenib. The low cost, easy determination, and reproducibility of a full blood count make the SII a promising tool for assessing HCC prognosis in future clinical practice.

P-0619

Comparison of different screening schemes for patients with hepatitis B associated cirrhosis

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Aim: To compare different screening schemes for patients with hepatitis B associated cirrhosis in terms of detection rate and impact on prognosis.

Methods: This retrospective study identified a total of 255 patients diagnosed with hepatitis B associated cirrhosis between January 2007 and January 2008 in Alimentary Diseases Centre of Beijing Youan Hospital. These patients were classified into the following two groups according to the time intervals at which they received serum AFP or ultrasonography examination: 82 cases for 3 mo (group A), and 173 cases for 6 mo (group B). The patients were followed for 4 years.

Results: A total of 33 out of 255 patients were detected with hepatocarcinoma (16 cases in group A, 17 cases in group B), and patients with hepatocarcinoma receiving continued monitoring for 2 years. Higher survival rate was detected in group A (81.3 %, 13/16) compared with group B (47.1 %, 8/17), but the difference was not statistically significant ($P = 0.071$). According to the Barcelona Clinic Liver Cancer (BCLC) staging standard, significantly more cases of early stage hepatocarcinoma were screened in group A (68.8 %, 11/16), compared with group B (5.9 %, 1/17) ($P = 0.000$).

Conclusion: Our research showed that screening at a 3-mo interval is associated with a higher detection rate of early stage hepatocarcinoma, increased radical cure opportunity and better survival rate for patients with hepatitis B associated cirrhosis.

P-0620

Efficacy of a new therapeutic assessment score (ACTH score) for advanced HCC patients receiving HAIC**Issei Saeki¹, Tsuyoshi Ishikawa¹, Taro Takami¹, Takahiro Yamasaki¹, Isao Sakaida¹**¹Department of Gastroenterology and Hepatology, Yamaguchi University Graduate School of Medicine, Yamaguchi, Japan;²Department of Oncology and Laboratory Medicine, Yamaguchi University Graduate School of Medicine, Yamaguchi, Japan

Background and Objectives: Hepatic arterial infusion chemotherapy (HAIC) is a recommended treatment option for advanced hepatocellular carcinoma (HCC) in Japan. Because of the poor prognosis of HAIC non-responders, it is important to identify patients who may benefit from continuous HAIC treatment. Although HCC staging can predict patient prognosis, there are currently no assessment scores to aid decision-making with regard to continuous HAIC treatment. Therefore, we established a new therapeutic assessment score for such patients and investigated its efficacy.

Methods: We analyzed 90 advanced HCC patients with elevated baseline alpha-fetoprotein (AFP) and/or des-gamma-carboxy prothrombin (DCP) levels and evaluated various parameters for their possible use as survival predictors. AFP and DCP responses were assessed after half a course of HAIC (2 weeks); a positive-response was defined as a reduction of >20 % from baseline. Furthermore, we investigated the efficacy of the established score in 40 patients.

Results: Child-Pugh (HR 1.99, $P = 0.018$), AFP response (HR 2.17, $P = 0.007$), and DCP response (HR 1.90, $P = 0.030$) were independent prognostic predictors. Considering these 3 factors, we developed an Assessment for Continuous Treatment with HAIC (ACTH) score (0–3 points). Patients stratified into two groups according to their ACTH score showed significantly different prognoses (<1 vs. >2 points, $P = 0.003$). Significantly different prognoses (<1 vs. >2 points, MST 21.7 vs. 9.8 months, $P = 0.048$) were also observed in the validation study.

Conclusions: The ACTH score aids in the therapeutic assessment of advanced HCC patients receiving HAIC.

P-0621

E-PASS scoring system is a good predictor of prognosis in HCC patients underwent major hepatectomy**Yuki Kitano, Akira Tsuji, Risa Inoue, Kensuke Yamamura, Takayoshi Kaida, Kota Arima, Takaaki Higashi, Katsunori Taki, Hidetoshi Nitta, Daisuke Hashimoto, Akira Chikamoto, Takatoshi Ishiko, Toru Beppu, Hideo Baba**

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Background: As complications after major hepatectomy are often serious, the adoption of an operation should be decided carefully. Although various scoring systems have been considered as useful predictors of prognosis, there is no report that shows the usefulness of a scoring system for the prediction of prognosis after major hepatectomy. We investigated the usefulness of the E-PASS scoring system as a predictor of short and long-term prognosis in HCC patients who underwent major hepatectomy.

Method: E-PASS predictor equations were applied retrospectively to 70 patients who had undergone major hepatectomy for HCC between 2008 and 2013. We calculated the Comprehensive Risk Score (CRS)

using the E-PASS scoring system and examined whether CRS had a correlation with the occurrence of postoperative complications (Clavien-Dindo>IIIa) and survival rate.

Result: Of the 70 patients, 20 (28.6 %) developed postoperative complications. The CRS value of complication group was significantly higher than that of the no complication group (0.77 ± 0.57 vs. 0.38 ± 0.19 , $P < 0.0001$). According to multivariate logistic regression analysis, the high CRS value (>0.5) could be identified as an independent predictive factor of the occurrence of postoperative complications (OR 9.5, $P = 0.007$). In terms of long-term prognosis, as a result of the survival curve using Kaplan–Meier, the three years survival rate of the high CRS group tended to be lower than that of the low CRS (<0.5) group (high CRS 44.9 % vs. low CRS 81.3 %, $P = 0.2$).

Conclusion: According to our results a high CRS value is a risk factor of postoperative complications in HCC patients who underwent major hepatectomy.

P-0622

Korean validation and comparison of prognostic scores for transarterial chemoembolization**Hyun Yang, Si Hyun Bae, Soon Kyu Lee, Ji Won Han, Hae Lim Lee, Jeong Won Jang, Jong Young Choi, Seung Kew Yoon**

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Background/aims: Transarterial chemoembolization (TACE) is one of the treatment options for unresectable hepatocellular carcinoma (HCC). There are four prognostic scores for TACE: ART, ABCR, HAP and modified HAP (mHAP). However, there is lack of validation of these scores, especially in Korea. The aim of this study is to validate and compare these scores.

Methods: From October 2008 to February 2014, total of 1211 patients with HCC were treated with TACE in Seoul Saint Mary's Hospital. Among them, 800 patients underwent at least two sessions of TACE within 90 days. 549 patients were excluded according to exclusion and inclusion criteria. Finally, total of 251 patients were analyzed. The validation in ART, ABCR, HAP, and mHAP score was performed and these scores were compared using the AUROC curve. **Results:** Mean age was 59.2, median follow up period was 24.2 months and median cycles of TACE was four. From these scores, OS of each group was calculated: ART (63.7, 37.7 months in 0–1.5, ≥ 2 , $P = 0.142$), ABCR (57.6, 18.1, 7.9 in ≤ 0 , 1–3, ≥ 4 , $P < 0.001$), HAP (60.2, 52.2, 20.8, 10.8 in 0, 1, 2, >2, $P < 0.001$), mHAP (58.7, 48.7, 16.6, 7.9 in 0, 1, 2, >2, $P < 0.001$). And the AUROC of survival was calculated (0.508, 0.709, 0.662, 0.652 in 1 year and 0.498, 0.679, 0.654, 0.650 in 2 years, in ART, ABCR, HAP, mHAP, respectively).

Conclusions: In our study, ABCR, HAP and mHAP were well applicable for prediction of prognosis of TACE. However, ART was not applicable. According to the comparison of these scores, ABCR was best predictable prognostic score for TACE.

P-0623

Impact of Antiviral Therapy on Hepatitis C Patients After Curative Hepatocellular Carcinoma Therapy**Ching-Sheng Hsu¹, You-Chen Chao¹, Hans Hsienhong Lin¹, Ding-Shinn Chen², Jia-Horng Kao²**

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Background: Hepatitis C virus (HCV) is the leading cause of hepatocellular carcinoma (HCC), and several antiviral agents are available for the treatment of chronic HCV infection. However, the impact of antiviral therapy on the long-term outcomes of HCV-related HCC patients remains inconclusive.

Aims: We aimed to examine the impact of antiviral therapy on the long-term outcomes of HCV-related HCC patients. **Methods:** We conducted a systemic review using PRISMA guidelines to identify trials and English-language literature from PubMed, Ovid MEDLINE, Scopus and the Cochrane Library database till August 2014. Randomized trials of antiviral treatments examining the effects of antiviral therapy on HCV-related HCC patients were screened and selected.

Results: We identified 6 trials evaluated the effectiveness of interferon (IFN)-alpha treatment, 3 studies examined pegylated interferon-alpha treatment, and 2 studies examined IFN-beta treatment. IFN-based therapy was associated with the improvement of liver reserve, decrease of HCC recurrence rate, and increase of survival rate in HCV-related HCC patients after curative HCC therapy.

Conclusions: This systemic review supports the beneficial effects of IFN-based treatment for decreasing HCC recurrence rate, improving serum ALT levels and liver reserve in HCV-related HCC patients after curative HCC therapy. However, future studies are required to clarify the impact of directly acting antivirals (DAAs) on HCC risk and cirrhotic complications in chronic hepatitis C patients and HCV-related HCC patients after curative HCC therapy.

P-0624

SVR of interferon-based therapy as a prognostic factor for HCV-related hepatocellular carcinoma

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Aim: Whether an anti-viral interferon (IFN)-based therapy (IBT) after curative treatment of hepatocellular carcinoma (HCC) improves the prognosis in patients with HCV-related HCC remains to be elucidated.

Methods: A total of 178 patients within the Milan criteria underwent curative treatment for HCV-related HCC. Both the time to beyond the Milan criteria (TTBMC) and overall survival (OS) were compared between the sustained virologic response (SVR) (IFN with SVR,

n = 22), non-SVR (IFN without SVR, n = 19), and non-IBT (control, n = 82) groups using propensity score matching analysis. Prognostic factors to predict survival were also determined by the Cox proportional-hazards model.

Results: TTBMC in the IFN with SVR group was significantly longer than those in the control and IFN without SVR groups (p < 0.001 and p = 0.006, respectively), although no significant difference existed between the IFN without SVR and control groups. Similarly, OS of the IFN with SVR group was significantly longer than that of the control and IFN without SVR groups (p < 0.001 and p = 0.029, respectively), although no significant difference existed between the IFN without SVR and control groups. The Cox proportional-hazards model identified SVR as an independent prognostic factor in these patients. The IFN with SVR group showed a 0.096-fold decrease in mortality risk compared with the control group (95 % confidence intervals = 0.023–0.405; p = 0.001).

Conclusion: Elimination of HCV after curative treatment of patients with HCC within the Milan criteria inhibits recurrence and contributes to a preferential prognosis.

P-0625

Influence of HBV DNA on recurrence of HCC after surgical resection and role of antiviral therapy

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Aims: To investigate the influence of HBV DNA elevation on HCC recurrence and the preventive role of antiviral therapy.

Methods: One hundred forty four patients who had BCLC stage 0 or A and received surgical resection as primary therapy were enrolled. HBV DNA elevation was defined as reactivation in patients without preoperative antivirals or virologic breakthrough in patients with preoperative antivirals.

Results: Overall 1, 3, 5 year recurrence was 20.1, 33.3, 45.1 %. In multivariate analysis, multiple tumor, HBV DNA elevation, ALT ≥ 30 , were independently associated with HCC recurrence. To rule out the antiviral effect, we investigated risk factors for HCC recurrence in each group with or without preoperative antivirals. In both groups, multiple tumor and HBV DNA elevation were independent risk factors for HCC recurrence. In multivariate analysis, age < 50 years, no preoperative antivirals, HBV DNA ≥ 2000 IU/mL were risk factors for HBV DNA elevation. Risk factors for HBV DNA elevation were further analyzed in patients without preoperative antivirals. Age < 50 years, HBV DNA ≥ 2000 IU/mL, HBeAg positivity and delayed antiviral therapy were independent risk factors for HBV DNA elevation.

Conclusion: HBV DNA elevation after resection increases the risk of HCC recurrence irrespective of preoperative antivirals. Delayed antiviral therapy after HBV elevation was associated with higher HCC recurrence compared to preoperative antiviral therapy. Therefore, perioperative antivirals should be considered to prevent HBV DNA elevation and recurrence, especially in patients with age < 50 years and/or HBV DNA ≥ 2000 IU/mL and/or HBeAg positivity.

P-0626

Radiofrequency Ablation in very elderly patients (85 years old or older) with liver tumors

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HCC increases in elderly patients. Some cases are often seen that very elderly patients (VEP 85 years old or older) are not targeted for positive treatment of HCC because of reduced hepatic function, hypertension, diabetes, dyslipidemia, and functional disorders of other organs. Choosing RFA in VEP with HCC must be decided by considering radicality and tolerability. We aimed to evaluate the safety and efficacy of RFA in VEP in this study. 34 patients were underwent RFA between December 2012 and November 2014 at our institute. We investigated the rate of cases which were beyond the general indication of RFA, 3 or fewer nodules, all 3 cm or less in diameter, the rate of complications, technical success rate, and 1-year survival. A total of 55 RFA treatments were performed in 34 VEP. In 30 HCC patients, 8 of 45 treatments were beyond the general indication. Grade II complications occurred in 1 of 45 (intraperitoneal bleeding required blood transfusion). Technical success rate was 100 % and 1-year survival was 90 %. In 4 metastatic liver cancer patients, 3 of 8 treatments were beyond the general indication. Complications did not occur. Technical success rate was 100 % and 1-year survival was 100 %. RFA was performed safely in VEP. Short-term efficacy judged by technical success rates and 1-year survival was satisfactory, although there were many patients beyond the general indication of RFA. RFA may be a treatment of choice in VEP not only with HCC but also with metastatic liver tumors.

P-0627

Usefulness of radiofrequency ablation for the elderly with hepatocellular carcinoma

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Background: The number of elderly patients who receive treatment for hepatocellular carcinoma has been increased. Usefulness of ultrasound-guided radiofrequency ablation (RFA) as for the prognosis of elderly patients has not been fully understood.

Methods: Survival after the treatment was compared between the elderly patients (80 years or older of age) and the non-elderly who received surgical resection or RFA as the initial treatment for hepatocellular carcinoma (HCC) between 2002 and 2014.

Results: Nine-hundred-seventy-five patients, comprising 680 male and 295 female at the age of 70 years in average, were retrospectively evaluated for their survival after the treatment. Among the elderly, 37 patients were treated with resection, and 80 patients with RFA, while 431 patients with resection and 427 patients with RFA among the

non-elderly. Among the elderly, the resected patient group had significantly better scores of Child-Pugh, JIS, and CLIP before the treatment than the RFA-treated patient group. Cumulative survival rates were significantly better in the RFA-treated patient group than resected patient group with the rates of 72 and 48 % at 3 years after the treatment, and those of 40 and 30 % at 5 years, respectively ($p = 0.037$). In comparison between the elderly and the non-elderly among the RFA-treated patients, cumulative survival rates were comparable between the groups, although the scores of Child-Pugh, JIS, and CLIP were significantly better in the elderly group.

Conclusions: Survival after the treatment might be better with RFA than surgical resection for the elderly patients with HCC.

P-0628

Percutaneous radiofrequency ablation for hepatocellular carcinoma located in the caudate lobe

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Aim: This study aimed to evaluate the effectiveness and safety of radiofrequency ablation (RFA) for hepatocellular carcinoma (HCC) located in the caudate lobe of the liver.

Patients and methods: Between 2012 April and 2014 February, 142 patients with HCC meeting the Milan criteria were enrolled in this study. Of these patients, nine patients had HCC located in the caudate lobe (caudate group). Six of the nine cases were located in the Spiegel lobe, two cases were located in the paracaval portion and one case was located in the caudate process. We evaluated the local recurrence rate and RFA-related complications in the caudate group and non-caudate group.

Results: The local recurrence rate in the caudate group was 12.5 % at 1 year and 12.5 % at 2 years, while the local recurrence rate in the non-caudate group was 14.9 % at 1 year and 29.0 % at 2 years; there were no significant differences between the groups. No complications were observed in the caudate group, and minor complications were observed in six patients (4.5 %) in the non-caudate group. No major complications or mortalities were observed in either group, and the complication rates were not significantly different between the groups ($P = 1$).

Conclusions: RFA for HCC in the caudate lobe and the non-caudate lobe has equivalent effectiveness and safety. RFA is a promising treatment option for HCC arising in the caudate lobe.

P-0629

Usefulness of 4D us to evaluate effects therapeutic RFA for HCC

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Introduction: In the present study we investigated whether 4D realtime flow imaging is useful to display the accurate position of percutaneous RFA needle in the tumor and evaluated the efficacy of RFA therapy in patients with HCC.

Materials and methods: 58 patients admitted to our Masuko Memorial Hospital All patients enrolled showed hypervascular enhancement of HCC on dynamic CT All patients gave written informed consent and this protocol had been approved by the Human Studies Committee at Masuko Memorial Hospital US imaging We used VOLUSON730 GE Medical systems APLIO XG Toshiba Medical Systems and IU22 Phillips for RFA therapy with a convex probe as US system. 4D Realtime refers here to the display of 3-dimensional moving images composed of 3 orthogonally intersecting scans in the transverse longitudinal and horizontal planes.

Results: We confirmed by various angles that the needle was inserted into the center of tumor nodule The simultaneous study before RFA therapy showed the inflow of arterial blood and tumor stain And importantly it appeared that 4D realtime US provided much perceptible information on the spatial relationship between RFA needle and the target lesion and resulted in accurate therapeutic efficacy for percutaneous RFA procedure.

Conclusion: We experienced the treatment of 58 patient with HCC by RFA using 4D realtime ultrasound system Application of this method allowed a more accurate cauterization of the tumor

P-0630

Evaluation of laparoscopic multipolar radiofrequency ablation for localized hepatocellular carcinoma

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Background: Radiofrequency ablation (RFA) has used widely as locoregional therapy for hepatocellular carcinoma (HCC). We have performed laparoscopic RFA (LRA) using a multipolar RFA system for treatment of HCCs since 2014.

Methods: We performed LRA in patients within Milan criteria (1 lesion < 5 cm, or 2–3 lesions each < 3 cm). Needle applicator was inserted under laparoscopic ultrasonography guidance, regardless of tumor location. It aimed at the parallel insertions based on ‘dosimetry table’ and no-touch ablation as much as possible.

Results: Sixty-eight patients with 114 HCCs were treated by multipolar LRA. The median size of main tumor was 22 mm (range, 10–42). The median follow-up time was 9.0 months (2.2–19.7). In all cases, sufficient ablative area as we planned was obtained. All patients have stayed alive and there was no procedural complication and local recurrence, while intrahepatic recurrence in the other segment was occurred in five patients.

Discussion: The laparoscopic approach enables parallel insertion of multiple applicators without limitation by echo window and/or the ribs. Especially in case of HCC in hepatic surface, it is highly useful to avoid thermal injury to adjacent organs by maintaining the space with pneumoperitoneum and immersion. In the short-term results, local recurrence has not occurred. To place applicators to the periphery of the tumor as possible with intention to no-touch ablation might lead to obtain a sufficient safety margin.

Conclusions: Although multipolar LRA required some proper skills, it is efficacious in treatment for localized HCCs by gaining good ablative area safely.

P-0631

Hepatocellular carcinoma tumour ablation procedures: less is more ?

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Background and aims: This randomised-controlled trial compares clinical efficacy of combining transarterial chemoembolism (TACE) and switch-control radiofrequency (SWC-RFA) versus SWC-RFA alone for hepatocellular carcinoma with size of 3.1–7cm.

Methods: The primary aim is to examine the clinical outcome in terms of primary technical effectiveness, overall survival analysis and progression free survival. Hospitalization days, re-admission rate and major complications were also reviewed.

Results: 29 patients were recruited into the combination TACE/SWC-RFA cohort (mean tumor size 4.51 cm, maximum 6.7 cm) and 26 patients into the SWC-RFA only cohort (mean tumor size 4.18 cm, maximum 6.2 cm). No statistically significant difference was noted in clinical outcome, with both groups achieving near 90 % complete remission. Both cohorts recorded clinical complications with more major complications observed in the combination TACE/SWC-RFA cohort. At the end of the study, mortality rate was 7 patients (26.92 %) in the SWC-RFA cohort and 4 patients (13.33 %) in the combination TACE/SWC-RFA cohort. Log rank test on overall survival analysis revealed no significant difference between the two groups ($P = 0.4751$). The tumor free survival at 6 months, 1 and 2 years was SWC-RFA only at 84, 68, 48 % and combination TACE/SWC-RFA at 79.31, 55.17, 31.03 %—($P = 0.1682$).

Conclusions: The study demonstrated equivalent clinical outcome between TACE/SWC-RFA and SWC-RFA only cohort. However combination TACE/SWC-RFA therapy was associated with more severe clinical complications. Hence SWC-RFA alone may be a sensible alternative treatment procedure in HCC with mean tumor size of 4 cm and perhaps larger size with various ablation outcome.

P-0632

Radiofrequency ablation by new adjustable electrode: initial experience

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VIVARF system (STARmed, Korea) became available in Japan in 2015. This system has an internally cooled electrode in which we can adjust the length of active tip from 0.5 to 3 cm. We first did ex vivo studies of bovine livers in March. The coagulation zone was almost sphere and its ablation volume changed in accordance with the length of active tip. Next day, we performed RFA using VIVARF system on 5 cases. We found that the electrode tip was too hyperechogenic. If the tip of the electrode is not detected correctly by ultrasound, we cannot use it in the treatment of tumors located in risky places, such as near vessels. We requested them to adjust its echogenicity. They provided 5 types of improved electrodes in May. We performed RFA

using them on 5 cases. Although the echogenicity of the electrode tip became better, it was not satisfactory yet. We requested further improvement. They brought 6 kinds of newly modified electrodes in July. A phantom experiment showed that the tip of at least one electrode was detected by ultrasound as precisely as that of the conventional cool-tip electrode. We performed RFA on 6 cases using the most appropriate electrode in August. The ultrasound image of the electrode tip was satisfactory. Through two times of improvements, the visibility of the electrode is now comparable to the conventional internally cooled electrode. New adjustable RF electrode is more cost-effective in some cases and may have more potential than fixed tip electrodes.

P-0633

Ten-year outcomes of radiofrequency ablation for hepatocellular carcinoma

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Background and aim: Liver functional reserve is crucial in determining the prognosis of hepatocellular carcinoma (HCC) patients. This study aimed to investigate 10 year outcomes of radiofrequency ablation (RFA) for HCC with a new prognostic grade, the albumin bilirubin (ALBI) grade.

Methods: We enrolled 622 HCC patients who underwent RFA at Taipei Veterans General Hospital from 2002 to 2013. Prognostic factors were analyzed in terms of overall survival and recurrence. The ALBI grade was calculated as $\pm 0.085 \times (\text{albumin g/l}) + 0.66 \times \log (\text{bilirubin } \mu\text{mol/l})$ and compared with other prognostic factors in prediction of overall survival.

Results: The 5 and 10 year overall survival rates were 63.1 and 48.7 %, respectively. The cumulative rate of recurrence at 5 and 10 years were 74.2 and 83.5 %, respectively. Factors associated with overall mortality were age >65 years ($p < 0.001$), prothrombin time international normalized ratio >1.1 ($P = 0.030$), alphafetoprotein (AFP) >20 ng/ml ($P = 0.014$), Barcelona Clinic Liver Cancer (BCLC) stage B ($P = 0.011$) and ALBI grade 2 & 3 ($p < 0.001$). Besides, multivariate analysis disclosed that age >65 years ($P = 0.018$), alanine aminotransferase >40 ($P = 0.021$), AFP > 20 ng/ml ($P = 0.001$), multinodularity ($p < 0.001$) and tumor size >2 cm ($P = 0.001$) predicted higher incidence of developing recurrence after RFA. Furthermore, ALBI grade had a good predictive accuracy for overall survival in comparison to other prognostic factors.

Conclusions: Ten year survival outcomes of hepatocellular carcinoma after radiofrequency ablation were excellent. The ALBI grade provided a feasible marker for predicting the prognosis of HCC patients undergoing RFA.

P-0634

The risk factors for percutaneous radiofrequency ablation under monitored anesthesia care

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Aims: Radiofrequency ablation (RFA) is a useful procedure for treating hepatic malignancies. RFA sometimes causes severe pain, which increases demand for monitored anesthesia care (MAC). We aimed to evaluate safety and efficacy of MAC during RFA in this study.

Methods: 371 patients underwent RFA under MAC between December 2014 and July 2015 at our institute. RFA is performed under deep intravenous sedation. Factors relevant to respiratory depression and severe body movement were retrospectively investigated. The respiratory depression was defined as oxygen saturation <90 %, and applying the triple airway maneuver, and we severe body movement as movement caused by pain, in which we should lower power of the generator.

Results: 341 patients were eligible. There occurred 47 respiratory depressions and 102 severe body movements. The risk factors for severe body movement by the multivariate analysis were number of puncture (OR: 2.06, $p < 0.0001$), number of tumor (OR: 1.79, $p < 0.0001$), metastatic liver tumor (OR: 3.66, $p = 0.0016$), dyslipidemia (OR: 0.17, $p = 0.0009$), and pentazocine (OR: 1.03, $p = 0.0009$). The risk factors for respiratory depression by the multivariate analysis were number of puncture (OR: 1.22, $p = 0.017$), number of tumor (OR: 1.60, $p = 0.0012$), hypertension (OR: 0.37, $p = 0.0072$), and pentazocine (OR: 1.02, $p = 0.04$).

Conclusions: A large number of puncture and tumor are the independent risk factors for severe body movement and respiratory depression with MAC during RFA. Also, metastatic liver tumor is the independent risk factor for severe body movement.

P-0635

OK432-stimulated monocyte-derived dendritic cell injection into HCC after RFA

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Background: Dendritic cell (DC)-based immunotherapies are believed to contribute to the eradication of the residual and recurrent tumor cells including hepatocellular carcinoma (HCC). The present study was designed to assess the safety, bioactivity and clinical response of OK432-stimulated MoDCs infusion into HCC following radiofrequency ablation (RFA), which is radical and curative treatment.

Methods: MoDCs were derived from 30 HCC patients in the presence of IL-4 and GM-CSF for 5 days. The cells were cultured for two additional days in the medium and stimulated with 0.1 KE/ml OK432. On day 7, DCs were harvested for injection, 5×10^6 cells suspended in 5 ml normal saline containing 1 % autologous plasma, and injected into HCC with a needle percutaneously after RFA. A group of 20 patients treated with RFA without DC administration were enrolled as a control. Adverse events and clinical response were monitored after DC infusion for two years.

Results: There were no grades III or IV National Cancer Institute Common Toxicity Criteria adverse events. Recurrence free survival in the patients treated with RFA and OK432-stimulated DC transfer was much better than that of controls ($P = 0.047$). Furthermore, a multivariate analysis showed that primary HCC group ($P = 0.029$) was associated with improved recurrence free survival.

Conclusions: DCs infusion subcutaneously following RFA did not cause additional adverse events. These results suggest that OK432-stimulated DC with curative treatments may enhance anti-tumor response against primary HCC.

P-0636

The benefit of balloon occluded TACE compared with conventional TACE for BCLC stage B HCC patients

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Background: To investigate the efficacy of balloon occluded TACE (bTACE) by using micro balloon catheter for patients with hepatocellular carcinoma (HCC) in BCLC stage B, we compared the treatment outcomes between bTACE and conventional TACE (cTACE).

Methods: We reviewed a total of 88 consecutive Barcelona Clinic Liver Cancer stage B HCC patients who had received first TACE in the period April 2012 to March 2015. 40 received bTACE and 48 received cTACE. The time to local progression (TTLP), time to progression (TTP) and overall survival (OS) were compared between bTACE and cTACE using propensity score matching and log rank test. The factors that affect disease progression and survival were identified by Cox proportional hazards regression analysis.

Results: The median TTLP, TTP and OS of bTACE and cTACE were 364 vs. 232 days ($P = 0.009$), 224 vs. 189 days ($P = 0.634$), NA vs. 833 days ($P = 0.308$) respectively. By Cox's regression analysis, bTACE was one of the factors to predict local progression [hazard ratio (HR), 0.49; 95 % confidence interval (CI), 0.26–0.94; $P = 0.034$], but not the prognostic factor of survival.

Conclusions: bTACE seems to prevent local progression compared with cTACE, but additional survival benefit of bTACE is still unknown.

P-0637

Double platinum therapy TAI and TACE for BCLC B hepatocellular carcinoma

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Background: TACE is the standard therapy for Barcelona Clinic Liver Cancer (BCLC) classification intermediate stage B hepatocellular carcinoma (HCC). On the other hand, transcatheter methods such as TAI also have an important role in treatment for advanced hepatocellular carcinoma (HCC). Although it has been reported that TAI with a high concentration of a fine-powder formulation of cisplatin (DDP-H) reduced intrahepatic recurrence and improved survival, there have yet to be any investigations into whether the combined use of TAI with DDP-H and TACE. We evaluated which TACE chemoagents in combination with DDP-H TAI contribute to survival in BCLC-B HCC.

Methods: Survival was analyzed in 55 patients who underwent DDP-H TAI and TACE for BCLC B HCC. The patients were divided into two groups; Epirubicin was used as the TACE chemoagent in 29 patients, and miriplatin was used in 26 patients.

Results: Survival time prolonged significantly in the miriplatin group compared with the epirubicin group. Multivariate analysis showed that Child-Pugh classification and up-to-7 criteria were factors that contribute to survival, and the selection of miriplatin as the TACE chemoagent was the treatment factor that most affected survival.

Conclusion: Double-platinum therapy with DDP-H TAI and miriplatin TACE will be a useful treatment strategy of improving survival for BCLC B HCC.

P-0638

Efficacy and safety of cisplatin vs doxorubicin in TACE for HCC

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Background: Transcatheter arterial chemoembolization (TACE) is one of the available treatment modalities for unresectable hepatocellular carcinoma (HCC). Doxorubicin or cisplatin is widely used as an anticancer agent for TACE, but it is still unclear which agent elicits a more favorable response. Here we compared the efficacy and safety of doxorubicin with those of cisplatin in the setting of TACE.

Patients: Thirty-three HCC patients scheduled for TACE were randomly assigned to a doxorubicin group ($n = 20$) and a cisplatin group ($n = 13$). The serum concentration of α -fetoprotein was measured at 0, 3 and 6 months, and tumor regression was evaluated at 6 months after TACE on the basis of CT findings.

Results: The two groups showed no differences in patient age, gender, number of tumors, tumor diameter, the serum concentration of α -fetoprotein, and liver function parameters including the Child classification. No serious adverse events such as abnormalities of hematological counts or renal dysfunction were observed in either of the groups. The serum concentration of α -fetoprotein was significantly decreased in the cisplatin group but increased in the doxorubicin group at 3 and 6 months after TACE ($p < 0.05$). The number of patients showing tumor regression was higher in the cisplatin group than in the doxorubicin group.

Conclusion: Cisplatin elicits a better response in terms of tumor regression than doxorubicin when used in TACE for HCC. Further studies are needed to evaluate the long-term effects of cisplatin as an anticancer drug for TACE in a larger cohort of patients.

P-0639

Interventional radiology for hepatocellular carcinoma in patients with Child-Pugh C

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Aim/background: Lack of donor in liver transplantation for hepatocellular carcinoma (HCC) has become a big issue. There is no consensus regarding whether interventional radiology for HCC in patients with Child-Pugh C liver cirrhosis will improve prognosis. To elucidate the effectiveness of such treatment, we evaluated the clinical features of affected patients.

Method/materials: Naive HCC with Child-Pugh C (n = 236) were enrolled (65.7 ± 10.2 years old, male:female = 170:66, Child-Pugh score 10:11:12: >12 = 92:66:48:30, HCV: HBV: HBV&HCV: alcohol: others = 151:41:2:12:30, ablative therapies:transcatheter arterial chemoembolization (TACE):chemotherapy:radiotherapy:liver transplantation (LT):best supportive care = 16:40:12:1:1:166]. Two of them were treated with LT after another treatments as bridging treatments (TACE and RFA). After exclusion of total 3 patients who received LT, we evaluated clinical factors related to improved prognosis, retrospectively.

Results: The percentage of all patients with total-bilirubin <3 mg/dL was 41.1 %. Prognosis of patients, who were received treatments (n = 30; ablative therapy 10, TACE 20) was better than non-treated of them (n = 18) (MST 22.2 vs. 13.8 months, P = 0.021, respectively) in patients within up-to-seven criteria and total-bilirubin <3 mg/dL (n = 48). On the other hand, there was no difference in prognosis between those who underwent ablative therapies (n = 10) and those who received TACE (n = 20) (MST 22.2 vs. 16.9 months, P = 0.390).

Conclusion: Therapy for HCC may prolong survival in patients with naive HCC, within up-to-seven criteria, and total-bilirubin <3 mg/dL.

P-0640

Response at first chemoembolization plays a favorable outcome in hepatocellular carcinoma

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Transarterial chemoembolization is the recommended treatment for patients with Barcelona Clinic Liver Cancer staging system stage B hepatocellular carcinoma. In this study, we try to assess factors determining good response (whether partial or complete response) at first TACE and outcome. From January 2010 to March 2014, 814 patients with newly diagnosed HCC were treated at the Chang Gung Memorial Hospital, Linkou branch, 519 patients underwent TACE with on-demand policy. We categorized patient as four groups according to modified Response Evaluation Criteria in Solid Tumors guideline. The median age was 63.1 years (54.9–72.3), 72.4 % were males and 86.9 % had HBV or HCV infection. Median overall survival from first TACE was 31.9 months (14.3–47.8). Overall, 296 patients could achieve response at first TACE. By multivariate analysis, CTP class A (OR: 2.38, 95 % CI 1.25–4.53, P = 0.008), within Milan criteria (OR: 1.86, 95 % CI 1.19–2.91, P = 0.006), tumor extent unilobar (OR: 1.94, 95 % CI 1.27–2.95, P = 0.002), no macrovascular invasion (OR: 2.79, 95 % CI 1.54–5.05, P = 0.001), AFP < 400 (OR: 1.59, 95 % CI 1.00–4.11, P = 0.049) and platelet < 150 thousand (OR: 1.58, 95 % CI 1.01–2.48, P = 0.045) are good predictors of approaching good response at first TACE. Patients who could have good response at first TACE had a higher probability to achieve complete response (55.4 vs. 9.9 %, p < 0.001) and better survival rate (3-years: 53.0 vs. 25.1 %, p < 0.001). In our study, good liver function, within Milan criteria, unilobar tumor extent, no macrovascular invasion, lower AFP and platelet level are the significant factors in determining good response at first TACE.

P-0641

The results of chemoembolization and endoarterial chemotherapy in primary hepatic carcinoma

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Purpose: Determination of results of chemoembolization and endoarterial chemotherapy in primary hepatic cancer. Was analysed the results in 108 patients. Tumor was localized in one part of liver in 42, in both parts in 66. Hepatocellular carcinoma was determined in 53, cholangiocellular carcinoma in 39, Hepatic hemangioendothelioma in 16 cases. Chemoembolization spent in 47 patients with doxorubicinum 60–80 mg and jodolypol with metallic spiral. In 61 patients we made endoarterial chemotherapy during 120 h. Chemo-toxicity after chemoembolization was 0 degree in 23.4 % patients, in 51.0 % 1st degree, in 25.5 % 2nd. After endoarterial chemotherapy in 45.9 % determined 0 degree of toxicity, in 37.7 % 1st and in 16.4 % 2nd degree. The partial regression of tumor size observed in 55.3 % and stabilization in 38.3 %, but in 6.4 % patients was progression. The regression was observed in all patients with Hepatic hemangioendothelioma and in 10 from 23 patients with Hepatocellular carcinoma. The toxicity results after endoarterial chemotherapy was better than after chemoembolization, but partial regression was increase at endoarterial chemotherapy in comparison with chemoembolization. We made puncture biopsy on 4 weeks after treatment in 31 with chemoembolization and in 37 patients with endoarterial chemotherapy. The 1st stage of pathomorphose was observed in 13 and 14 patients corresponded. The 2nd stage observed in 15 after chemoembolization and in 19 after endoarterial chemotherapy. Therefore analysis was shown that chemoembolization is method of choice in the treatment of Hepatic hemangioendothelioma.

P-0642

Two cases of Bile Duct Injury after TACE**Jingjing Zhou, Xijing Xu, Yao Wang, Xiaoyu Wen, Qinglong Jin**

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Transcatheter Arterial Chemoembolization has been widely accepted as a safe and effective treatment of primary and secondary malignant hepatic tumors. Despite the excellent therapeutic effects of TACE, a spectrum of complications occurs after TACE of hepatocellular carcinoma. Among these, complications related to bile duct injury have been reported including a biliary stricture; biloma; biliary peritoneum; hemobilia and biliopleural fistulas. Here we report two cases of bile duct injury that develop after TACE of HCC. A 46-year old man developed intrahepatic biloma within 2 months of chemoembolization. A 53-year old man developed bile duct necrosis in 3 months after chemoembolization. They were admitted because of the epigastric discomfort and jaundice respectively. On CT, Intrahepatic biloma appeared as a round, solitary, or multiple cystic area and the bile duct necrosis appeared as bile duct vanishing. Because of the clinical symptoms, the patient with intrahepatic biloma was treated by percutaneous drainage and the patient with bile duct necrosis was treated with gallbladder drainage. The latter patients died of liver failure 2 months after discharge and another patient is in the follow up. According to the reference, in patients with hepatocellular carcinoma, there are potential risk factors of bile duct injury due to TACE. For the two cases, we think the technical-related risk factors such as proximal injection of drugs and repeated injection with a frequency of less than 3 months significantly influenced the incidence of bile duct injury.

P-0643

Chemoembolization with drug-eluting microspheres for hepatocellular carcinoma: short-term efficacy**Manabu Morimoto¹, Satoshi Kobayashi¹, Satoshi Moriya¹, Makoto Ueno¹, Shun Teduka¹, Kuniyasu Irie¹, Yoshihiro Goda¹, Shinichi Ohkawa¹, Toru Aoyama², Soichiro Morinaga², Kazushi Numata³, Katsuaki Tanaka³, Shin Maeda⁴**

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Purpose: To evaluate the safety and short-term efficacy of transarterial chemoembolization using superabsorbent polymer (SAP) microspheres loaded with epirubicin for the treatment of hepatocellular carcinoma (HCC).

Patients and methods: Consecutive 65 patients with unresectable HCC were treated by chemoembolization using SAP microspheres during the years 2013-2015. The average age of the patients was 72 years old, and their background liver diseases were hepatitis C virus and hepatitis B virus in 21 cases (32 %) and 8 cases (12 %), respectively. Child-Pugh A and B were included in 44 cases (67 %) and 21 cases (33 %), respectively. Maximum diameters of the

tumor were 47 mm, and 52 % of all the patients had more than 2 tumors. Tumor response was assessed by modified Response Evaluation Criteria in Solid Tumors with computed tomography (CT) or magnetic resonance imaging obtained 1 month after chemoembolization.

Results: Major complication was experienced in 4 cases (6 %), having post embolization syndrome requiring extended stay. Complete response and partial response were obtained in 6 cases (9 %) and 29 cases (45 %), and objective response rate was 54 %. The overall 1-year survival rate was 72.9 %. Univariate analysis showed tumor number, serum level of alpha-fetoprotein, serum level of des-gamma-carboxy prothrombin, and intra-procedural cone-beam CT findings were the response predictors.

Conclusions: Chemoembolization with SAP microspheres demonstrated a safety profile and good short-term effectiveness. Intra-procedural assessment by cone-beam CT might have an ability of instant monitoring the endpoint of chemoembolization.

P-0644

Clinical outcomes of DEB-TACE versus conventional TACE in hepatocellular carcinoma**Yong Kang Lee¹, Kyu Sik Jung^{1,2}, Seung Up Kim^{1,2,3}, Beom Kyung Kim^{1,2,3}, Jun Yong Park^{1,2,3}, Sang Hoon Ahn^{1,2,3,4}, Kwang-Hyub Han^{1,2,3,4}, Jin Young Choi⁵, Man Deuk Kim⁵, Meyong-Jin Kim⁵, Sung Il Park⁵, Jong Yoon Won⁵, Do Yun Lee⁵, Do Young Kim^{1,2,3}**

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Introduction: Doxorubicin eluting beads (DEB) invented to deliver higher doses of drug to hepatocellular carcinoma (HCC) in safe. To show efficacy and safety of DEB-TACE, we compared clinical outcomes of DEB-TACE to those of conventional TACE.

Methods: Total of 122 consecutive patients who underwent DEB-TACE and 179 consecutive patients underwent cTACE between January 2010 to April 2014 were retrospectively studied. The primary endpoint was overall survival (OS). The secondary endpoint was radiologic response and progression free survival (PFS). Radiologic response evaluated in 1 and 3 month by mRECIST criteria.

Results: Objective response rate, summation of complete and partial response rate, was significantly higher in cTACE at 1 month (80.3 vs. 90.0 %, $P = 0.015$) but better in DEB-TACE at 3 months follow up (82.9 vs. 48.1 % $P = 0.001$). The estimated mean OS were not differ between two groups ($P = 0.685$). OS of DEB-TACE was 47.4 months compared to 46.7 months in cTACE. The median PFS was better in cTACE (10.8 months in DEB-TACE vs. cTACE 13.3 months; $P = 0.007$). In patients with intermediate stage, estimated mean OS and median PFS was also not significantly different between two groups [OS (42.7 vs. 41.5 months; $P = 0.725$)], and PFS (12.5 vs. 14.2 months; $P = 0.428$), respectively.

Conclusion: DEB-TACE seems to be a safe and equivalent treatment modality in treating HCC even compared to cTACE. Although PFS was better in cTACE group, but there was no significant differences in OS. Furthermore patients diagnosed HCC compatible with intermediate BCLC stage, OS and PFS were not significantly different.

P-0645

Long-term survival results of HCC patients after Drug-eluting beads TACE**Ky Doan Thai¹, Bang Hong Mai¹, Thanh Tien Nguyen¹, Truong Van Le¹, Thong Minh Pham², Khien Van Vu¹**¹The Department of Hepato-Gastroenterology, Military Central Hospital 108, Hanoi, Vietnam; ²Bach Mai Hospital, 78 Giai Phong Street, Dong Da District, Hanoi, Vietnam**Aims:** to evaluate the long-term result of HCC patients treated with TACE using doxorubicin-loaded microspheres, and analysis some prognostic factors.**Subjects and methods:** a prospective non-randomized study was done on 105 HCC patients (mean tumor size: 7.8 ± 2.5 mm) undergoing TACE with drug-loaded beads at the 108 hospital, from June 2011 to February 2015. We used 1 or 2 from 3 different sizes of DC-Beads (100–300 μ , 300–500 μ and 500–700 μ) loaded with 50–150 mg doxorubicin in a procedural session. Survival was calculated from the date of first TACE, using Kaplan–Meier estimations. Log-rank test was used to analyze the differences in the mean survival time and the 1-, 2- and 3-year overall survival rates of subgroups according to prognostic factors.**Results:** 105 HCC patients underwent totally 198 TACE procedural sessions. The mean time of follow-up was 19.8 months. The mean overall survival time of all patients was 28 months (95 % CI 24–31). The cumulative survival rates at 1-, 2- and 3-year follow-up were 72.4; 55.2 and 41.3 %, respectively. Predictors for long-term survival were: Serum AFP, tumour morphology (single nodule or multinodules, mass or diffuse type), tumor size (smaller or larger than 8 cm), grade of tumour cell differentiation, vascular invasion, Child Pugh class, Okuda and Barcelona Clinic Liver Cancer staging.**Conclusions:** DC-Beads TACE is an effective treatment for HCC, with the clinical outcome depending on several prognostic factors.

P-0646

The short-term effectiveness of TACE using cisplatin-eluting HepaSphere for unresectable HCC**Takayoshi Oikawa, Youhei Kooka, Kei Sawara, Hidekatsu Kuroda, Yasuhiro Takikawa**

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Background: To evaluate the efficacy and safety of transcatheter arterial chemoembolization using cisplatin eluting HepaSphere (CEH-TACE) for unresectable hepatocellular carcinoma (HCC).**Methods:** CEH-TACE was performed using cisplatin 50 mg (IA-call : Nippon Kayaku Co., Japan) -eluting HepaSphere 50–100 μ m (Bio-Sphere Medical, Inc. USA) for 23 patients (male: 16, mean age 76.6 years, etiology: HBV/HCV/NBNC; 4/15/4, cStage: II/III/IV; 10/12/1, mean tumor diameter: 50.0 ± 24.3 mm) between April 2014 and September 2015. As the endpoint of the study, the efficacy and safety of the procedure were evaluated by modified Response Evaluation Criteria in Solid Tumors (m RECIST) with dynamic contrast-enhanced CT within 3 months post therapy, and Common Terminology Criteria for Adverse Events (CTCAE) ver. 4.0, respectively.**Result:** CEH-TACE was successfully performed in all patients. Partial response was achieved in 11 patients (Response rate: 52.2 %, Disease control rate: 73.9 %). Using CTCAE ver.4.0, no major adverse events occurred.**Conclusions:** CEH-TACE is a safe and effective treatment for unresectable HCC without adverse events.

P-0647

Comparison of the effects of TACE with T-ACE beads and other microspheres: a porcine study**Jui-Wen Kang¹, Chen-Hsi Chou², Yi-Sheng Liu³, Hong-Ming Tsai³, Hung-Wen Tsai⁴, Chiung-Yu Chen¹, Xi-Zhang Lin¹**¹Department of Internal Medicine, National Cheng Kung University Hospital, Tainan, Taiwan; ²Institute of Clinical Pharmacy and Pharmaceutical Sciences, National Cheng Kung University, Tainan, Taiwan; ³Department of Radiology, National Cheng Kung University Hospital, Tainan, Taiwan; ⁴Department of Pathology; National Cheng Kung University Hospital, Tainan, Taiwan**Background:** Hepatoma is one of the most common cancers worldwide. Most patients were diagnosed at intermediate stage. Transcatheter arterial chemo-embolization is recommended as first line treatment for these patients. We develop the microspheres to evaluate its safety and embolization effect in animal study.**Methods:** We developed new microsphere (T-ACE beads) consisted of pharmaceutical excipients that were generally regarded as safe or could be used as implants in human. The sizes of our microspheres were adjustable by sieving method, ranging 100–500 microns. We use T-ACE beads without doxorubicin (n = 8), or with doxorubicin (n = 9) as embolization material and compare with gelfoam (n = 8), embosphere (n = 8), DC beads (n = 6), and hepasphere (n = 6). Under anesthesia, the piglets were embolized via left/right hepatic and splenic artery by interventional radiologists. Serial blood tests and CT scan were performed to evaluate its efficacy and safety. All piglets were sacrificed at 7–35 days to get embolized liver and spleen tissue for histological examination.**Results:** 45 piglets were embolized with various embolization material. After sacrifice, all embolized material could lead to vasculitis, necrosis, and fibrosis in embolized region. Significant degradation of the T-ACE beads was also observed in pathological examinations. Transient elevation of WBC, GOT, GPT were noted at 24 h after embolization. Serial CT scan revealed shrinkaged spleen after embolization.**Conclusion:** Transcatheter arterial embolization with T-ACE beads loaded with or without doxorubicin is safe and effective comparing with current commercial microsphere.

P-0648

Bolus administration of CDDP with 5FU in hepatic arterial infusion chemotherapy for advanced HCC**Futa Koga¹, Taiga Otsuka¹, Kaori Gotanda¹, Norimasa Araki¹, Kenichiro Murayama¹, Hiroshi Isoda¹, Shunya Nakashita², Takumi Akiyama², Yasunori Kawaguchi², Yuichiro Eguchi¹, Seiji Kawazoe², Iwata Ozaki¹, Keizo Anzai¹**¹Department of Internal Medicine, Division of Hepatology, Saga University Hospital, Saga, Japan; ²Department of Hepatobiliary and Pancreatology, Saga-Ken Medical Centre Koseikan, Saga, Japan**Introduction:** Hepatic arterial infusion chemotherapy (HAIC) with 5-fluorouracil and cisplatin via an implantable port system is a therapeutic option for locally advanced hepatocellular carcinoma (HCC); however, adequate treatment schedule is still unknown. To determine

the optimal administration schedule of cisplatin for HAIC using 5-fluorouracil and cisplatin for patients with advanced HCC.

Methods: Consecutive patients with advanced HCC without extrahepatic metastasis who received the daily or bolus cisplatin regimens plus 5-fluorouracil of HAIC were retrospectively analyzed. All patients were diagnosed as ineligible for resection or locoregional treatment, or refractory to transarterial chemoembolization. The daily regimen consisted of 10 mg/body cisplatin on days 1–5 and 8–12, whereas the bolus regimen consisted of 65 mg/m² cisplatin on day 1. The both regimens followed by 5-fluorouracil 250 mg/body on days 1–5 and 8–12.

Results: Of the 128 patients deemed eligible, 80 received daily and 48 received bolus cisplatin. Their objective response rates (32.9 vs. 37.5 %, respectively; $P = 0.600$) and median time to progression (5.0 vs. 4.0 months; $P = 0.433$) were comparable, as was their median overall survival (OS) (13.0 and 13.4 months, respectively; $P = 0.549$). Of the 66 patients with macroscopic vascular invasion, 35 received daily and 31 received bolus cisplatin; their median OS was also similar ($P = 0.779$). Most treatment-related adverse events were similar in both groups.

Conclusions: There was no difference in the efficacy of the two regimens for locally advanced HCC. Bolus cisplatin administration is simpler than daily administration for patients.

P-0649

Efficacy of hepatic arterial chemotherapy of CDDP suspension in lipiodol and 5-FU for advanced HCC

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Purpose: To investigate which is better, Sorafenib or Hepatic arterial infusion chemotherapy (HAIC) in patients with advanced HCC with macroscopic vascular invasion (MVI), without extra-hepatic spread (EHS) and Child-Pugh class A disease, this multicenter none-randomized prospective cohort study was conducted.

Method: From April 2008 to March 2014, total 64 HCC patients with MVI, without EHS and Child-Pugh class A were registered. 44 were treated with HAIC, 20 were treated with Sorafenib. HAIC regimen comprised a combination of 50 mg fine powder formulation of Cisplatin in 5-10 ml lipiodol and continuous infusion of 5-fluorouracil (1500 mg/5 days). The primary endpoint was progression-free survival (PFS), while the secondary endpoints were Median survival time (MST), tumor response rate.

Results: There were no statistical differences in clinical factors between two groups. PFS in HAIC and Sorafenib was 9.3 and 4.1 months, respectively ($P = 0.002$). CR or PR rate in HAIC and Sorafenib was 71 and 10 % ($P < 0.001$). MST in HAIC and Sorafenib was 25.5 and 13.0 months, respectively ($P = 0.016$). Treatment-related mortality was not observed. Multivariate analysis revealed that independent predictor of survival were therapeutic effect (CR or PR, $P = 0.009$), Child-Pugh score (score 5, $P = 0.022$), grade of portal vein invasion (trunk, $P = 0.002$), and independent predictor of therapeutic effect was therapeutic regimen (HAIC, $P < 0.0001$).

Conclusion: In HAIC group PFS, MST and tumor response were significantly better than Sorafenib. This regimen should be the first choice for patients with advanced HCC with MVI, without EHS and Child Pugh A disease.

P-0650

Hepatic arterial infusion chemotherapy for Sorafenib Failure on advanced HCC

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We examined the effectiveness of the IFN/5FU hepatic arterial infusion as the second treatment in the case of SF(Sorafenib) failure.

Method: Subcutaneously interferon injection and 5FU hepatic arterial infusion (IFN/5FU) was treated for 26 cases from 2008 to 2012 in the cause of SF failure. 19 men, 7 woman, age 70 years old (24–79), tumor diameter 6.3 cm (0–16), tumor number 10 (2–30), stage III 7, IVa 9, IVb 10 (38 %). Portal vein tumor invasion positive was 17 patients (65 %). Child A was 21 patients. The protocol assumed 1 course only IFN for combination of IFN and 5FU, latter half 2 weeks for 2 weeks in the first half for 4 weeks. The effect measurement performed an image evaluation and tumor marker. The curative effect followed by RECIST criteria.

Results: The treatment effect was CR0, PR5 (19 %), SD14 (54 %), PD7 (27 %), and the response rate was 19 %. The disease control rate was 73 %. AFP was significantly decreased from 1547 to 810, DCP6689 to 4667. As for 17 cases that decreased with both AFP and DCP more than 20 %, it was admitted to (66 %) ($P = 0.03$) where the case that AFP decreased significantly accepted a convalescence improvement effect. MST after the IFN/5FU was 5.4 month. All survival MST from initial treatment was 4.6 years. There were no side effects of Grade 3 and the falling off.

Conclusion: In SF failure, the tumor marker was decreased to 66 %. The response rate was 19 %. However, it was useful as the second treatment in achieving MST 5.4 months in case of stage IVb was 38 %.

P-0651

Hepatic arterial infusion chemotherapy vs sorafenib for cTACE-refractory HCC

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Background: To investigate the antitumor efficacy of hepatic arterial infusion chemotherapy (HAIC) with cisplatin and 5-fluorouracil for conventional transarterial chemoembolization (cTACE)-refractory hepatocellular carcinoma (HCC), we compared the treatment outcomes between HAIC and sorafenib.

Methods: Between 1999 and 2012, consecutive patients with cTACE-refractory HCC without extrahepatic metastasis who had received sorafenib or HAIC were reviewed. Of the 97 patients deemed eligible, 30 received sorafenib and 67 received HAIC. The patients enrolled in this study were extracted using propensity score

matching, which calculated the selected variables influencing prognosis.

Results: Twenty-nine pairs of patients were successfully matched. In the sorafenib and HAIC groups, the objective response rates (7 and 34 %, respectively; $P = 0.012$) and median time to progression (2.0 and 4.2 months, respectively; $P = 0.009$) were favorable in patients who had received HAIC. The median survival times of the sorafenib and HAIC groups were 16.8 and 14.0 months, respectively ($P = 0.503$).

Conclusions: HAIC with cisplatin and 5-fluorouracil had promising antitumor efficacy relative to sorafenib, and could be an effective therapeutic option for patients with cTACE-refractory HCC.

P-0652

Hepatic arterial infusion chemotherapy with cisplatin and sorafenib in hepatocellular carcinoma

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Aim: We aimed to assess whether the clinical outcome and tolerability of hepatic arterial infusion chemotherapy (HAIC) using cisplatin as an alternative treatment to sorafenib for hepatocellular carcinoma (HCC) patients who had not responded to prior transarterial chemoembolization (TACE) in a multicenter cohort of the Kanagawa Liver Study Group.

Methods: Medical records of 127 consecutive HCC patients without extrahepatic metastasis (cisplatin: $n = 44$, sorafenib: $n = 83$) who had not responded to prior TACE at four institutions were retrospectively reviewed. An inverse probability of treatment weighting (IPTW) using propensity scoring was used to adjust for the selection bias. A non-inferiority margin was set as a 1.25.

Results: Severe adverse events accounting for treatment discontinuation occurred in 2.3 % of the patients in the cisplatin group and 32.5 % of those in the sorafenib group. The median overall survival was 11.2 months (95 % CI 4.8–17.7) in the cisplatin group and 10.2 months (95 % CI 8.8–11.5) in the sorafenib group, respectively. After an IPTW adjustment, overall survival of the cisplatin group was not inferior to that of the sorafenib group (hazard ratio 0.758; 95 % CI 0.471–1.219, $P = 0.253$). The upper limits of the 95 % CI was less than the noninferiority margin of 1.25.

Conclusion: HAIC with cisplatin could be an alternative treatment option in patients who have not responded to prior TACE, and it might be reasonable for the selection of HCC patients who are not able to tolerate sorafenib.

P-0653

Clinical evaluation of sorafenib treatment and HAIC for advanced HCC

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Aim: We aimed to evaluate prognostic factors of Sorafenib (SFN) treatment and hepatic arterial infusion chemotherapy (HAIC) for advanced HCCs.

Methods: We analyzed 72 patients treated with SFN and 128 patients receiving HAIC. Both treatment groups were statistically analyzed for prognostic factors at 1 month after starting of treatment and factors associated with post progression survival (PPS).

Results: Decrease of albumin ($< \times 0.8$) (HR 4.44, $P = 0.025$), deterioration of total bilirubin ($\geq \times 1.5$) (HR 1.97, $P = 0.027$) and Child-Pugh (CP) score (increase ≥ 2) (HR 9.52, $p < 0.001$) were poor prognostic factors at 1 month after the therapeutic start in the SFN group. Meanwhile, decline of PIVKA-II ($< \times 0.5$) (0.52, $P = 0.001$) and disease control (reduced or stable state in tumor size) (HR 0.18, $p < 0.001$) were good prognostic factors in the HAIC group. In both groups, CP score (≥ 8 points) (SFN; HR 3.72, $p < 0.001$, HAIC; HR 1.74, $P = 0.016$) was related to poor PPS, while subsequent treatment was long PPS factor (SFN; HR 0.11, $P = 0.001$, HAIC; HR 0.38, $p < 0.001$). Additionally, analyses of 53 patients selected from both groups based on the propensity score matching method showed no significant differences in survival between the two matched subgroups.

Conclusions: Deterioration of liver function was significantly correlated with poor prognosis at 1 month after starting the treatment in the SFN group, while response to treatment was strongly associated with prognosis in the HAIC group. The patients in both treatments should be subsequently and alternatively treated to obtain longer PPS if their liver function is preserved at the time of PD.

P-0654

Sorafenib enhances cytotoxicity via inhibition of cellular defense mechanisms

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Aim: Sorafenib has been reported to improve the prognosis of the patients with advanced hepatocellular carcinoma (HCC). However, the detailed mechanisms of sorafenib, especially direct effects to hepatoma cells and hepatocytes, are poorly understood. Therefore, more detail investigation about molecular mechanism of sorafenib seems to be necessary. The endoplasmic reticulum (ER) stress is related to the pathophysiology of various liver diseases, including chronic viral hepatitis, alcoholic and nonalcoholic steatohepatitis and HCC. Thus, we examined the molecular effects of sorafenib focused on the cellular defense mechanisms from ER stress.

Methods: We used human hepatoma cell lines (Huh7 and Hep3B) and a highly differentiated immortalized human hepatocyte cell line (OUMS29). The following reagents were used: sorafenib; acetyl-leucyl-leucyl-norleucinal (ALLN) and epoxomicin as proteasome inhibitors (PIs). Apoptotic cells were detected by TUNEL staining. Necrotic cells were detected by propidium iodide staining without membrane permeabilization. ER stress, oxidative stress, unfolded protein response (UPR), keratin phosphorylation and apoptosis were examined by Western blotting.

Results and Conclusion: The UPR and keratin phosphorylation are considered to be the representative cellular reaction induced by ER stress. Our data demonstrated that sorafenib inhibits the important

cytoprotective mechanisms and enhances cell death especially in combination with PIs. These observations may open the way to potentially interesting treatment combinations that may augment the effect of sorafenib, possibly including drugs that promote ER stress. Because sorafenib blocked the cellular defense mechanisms against hepatotoxic injury not only in hepatoma cells but also in hepatocyte-derived cells, we must be careful to avoid severe adverse effects.

P-0655

Sorafenib resistance can be overcome by Mfn2 through inhibiting HIF-1 α expression in HCC

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Sorafenib is an approved systemic drug of choice for advanced hepatocellular carcinoma (HCC), but has demonstrated limited benefits because of drug resistance. Hypoxia, one of the important tumor microenvironment, is association with multidrug resistance activated by enhanced hypoxia-inducible factor 1 α (HIF-1 α) expression. Mitofusin-2 (Mfn2), a novel functional tumor suppressor, plays a pivotal role in mitochondrial fusion, but its exact role in hypoxic chemotherapy resistance is poorly understood. We found that the expression of Mfn2 was decreased in HCC cells exposed to hypoxia compared with cells exposed to normoxia. Moreover, overexpression of Mfn2 can inhibit HIF-1 α protein accumulation in HCC, leading to an alleviative hypoxic tumor environment. Ectopic expression of Mfn2 could significantly recover sensitivity to sorafenib-induced apoptosis in hypoxic HCC cells and the synergistic tumor growth inhibition effects were also observed in subcutaneous hepatic tumors. In conclusion, Mfn2 overcomes hypoxia-mediated sorafenib resistance by inhibiting expression of HIF-1 α and the combination of Mfn2 overexpression and sorafenib represents a promising strategy for HCC.

P-0656

Efficiency of sorafenib in Japanese patients with hepatocellular carcinoma: a multicenter experience

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Introduction: Sorafenib is recommended for patients with advanced HCC. Patients after TACE-refractory or failure are also given sorafenib.

Methods: We analyzed 236 patients treated with sorafenib in five hospitals in Kagawa prefecture since June 2009 and evaluated by modified RECIST (189 men and 46 women; median age, 74 years old) to identify the characteristics that associated with response and survival.

Results: HCV infection (n = 133) was the predominant cause of liver disease, followed by HBV infection (n = 35), and alcohol consumption (n = 15). The disease in 184 patients (78 %) was rated as Child-Pugh class A at baseline. Sorafenib was administered because of TACE-refractory (n = 99), TACE-failure (n = 23), extrahepatic lesion (n = 84), or vascular invasion (n = 34). Seven patients (3 %) had a complete response, 22 (9 %) had a partial response, 90 (38 %) had a stable disease, and 113 (48 %) had a progressive disease. Logistic regression analysis demonstrated that dermatological adverse events (grade 2 or higher) and cardiac adverse events (grade 2 or higher) were associated with response [odds ratio, 3.02 (95 % CI 1.19–7.95) and 7.34 (2.22–24.62), respectively]. After stopping sorafenib, other therapies were given among 78 patients (33 %). Median overall survival was 406 days. Cox proportional hazards model demonstrated that the number of tumors less than 10 and performance status equal to 0 were associated with survival [risk ratio, 0.44 (95 % CI 0.30–0.65) and 1.54 (1.05–2.27), respectively].

Conclusions: Sorafenib was effective among patients with dermatological or cardiac adverse events. Meanwhile, small number of lesions and/or good performance status was associated with survival.

P-0657

Sorafenib for the treatment of advanced hepatocellular carcinoma with extrahepatic metastasis

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Background: Sorafenib, an oral multikinase inhibitor, is approved for advanced hepatocellular carcinoma (HCC) treatment. However, its therapeutic effect in advanced HCC patients with extrahepatic metastasis remains uncertain. This study aimed to prospectively assess the efficacy, safety, and prognostic factors and evaluate a survival impact of Sorafenib treatment in advanced HCC patients with or without extrahepatic metastasis.

Methods: Between May 2009 and March 2014, 312 consecutive advanced HCC patients who received sorafenib were enrolled in this study. We evaluated their characteristics, and compared the clinical outcomes of those with and without extrahepatic metastasis.

Results: Of the enrolled patients, 245 (81 %) patients could be received Sorafenib more than 1 month, and their median duration treated was 3.6 months. Eighteen patients demonstrated partial response to Sorafenib therapy, 127 had stable disease and 134 had progressive disease at the first radiologic assessment. The median survival time (MST) and progression-free survival (PFS) were 10.3 and 3.6 months, respectively. Multivariate analysis identified gender, Child-Pugh class, serum des-gamma-carboxy prothrombin levels and treatment duration as independent risk factors for survival. Of 245 patients evaluated, extrahepatic metastasis was detected in 178 patients, and the MST, PFS and therapeutic effect were studied between patients with and without extrahepatic metastasis. Negative prognostic factors on overall survival were similar regardless a presence of extrahepatic metastasis.

Conclusions: Our results indicated that Sorafenib could be administered regardless of their extrahepatic metastasis status, and hepatic function was the most important as for long-term treatment of patients with advanced HCC.

P-0658

Sorafenib might inhibit liver fibrogenesis in patients with cirrhosis and hepatocellular carcinoma**Daigo Matsui¹, Hidenari Nagai¹, Yu Ogino¹, Takanori Mukozu¹, Teppei Matsui¹, Michio Kogame¹, Noritaka Wakui¹, Koichi Momiyama¹, Mie Shinohara¹, Yoshinori Igarashi¹, Yasukiyo Sumino¹, Kazuhiro Matsuo², Koji Higai³**¹Division of Gastroenterology and Hepatology, Department of Internal Medicine, Toho University Medical Center Omori Hospital, Tokyo, Japan; ²Department of Medical Biochemistry, Faculty of Medical Pharmacology, Tokyo, Japan; ³Department of Medical Biochemistry, Faculty of Pharmaceutical Sciences, Toho University, Tokyo, Japan**Background:** It was reported that sorafenib (SF) might improve liver fibrosis and portal hypertension in an animal model.**Aim:** The aim of study was to clarify effects of SF on liver fibrosis in patients with liver cirrhosis (LC) and advanced hepatocellular carcinoma (aHCC).**Patients/methods:** One hundred one patients with LC and aHCC were treated with SF between 2009 and 2015. SF was administered at 400 mg/day for 4 weeks. Blood samples were collected before and after treatment to measure the serum concentration of the 7S domain of type IV collagen (CO), P-3-P (P), and hyaluronic acid (HA). Liver fibrosis was assessed by virtual touch tissue quantification (VTQ).**Results:** Twenty patients had HBV-related LC (group B), 53 patients had HCV-related LC (group C), and 28 patients had non-B, non-C LC (group N). Assessment of liver fibrosis showed that serum CO and P decreased significantly after treatment compared with before treatment in the group C and N, although there was no significant change in the group B. The medium VTQ value also decreased significantly after treatment compared with before treatment in the group C and N, although there was no significant change in the group B. Moreover, in patients without PVTT, the medium VTQ value decreased significantly after treatment compared with before treatment, although there was no significant change in patients with PVTT (Vp3<).**Conclusions:** Sorafenib might inhibit fibrogenesis in patients with HCV-related and non-B, non-C LC, although it might not inhibit fibrogenesis in patients with HBV-related LC.

P-0659

The improved prognosis of hepatocellular carcinoma with extrahepatic spread treated by sorafenib**Yoshinari Asaoka¹, Koji Uchino², Ryosuke Tateishi¹, Ryo Nakagomi¹, Mayuko Kondo¹, Naoto Fujiwara¹, Tatsuya Minami¹, Masaya Sato¹, Kenichiro Enooku¹, Hayato Nakagawa¹, Yuji Kondo¹, Kazuhiko Koike¹**¹The Department of Gastroenterology, University of Tokyo, Tokyo, Japan; ²JR Tokyo General Hospital**Background:** The systemic chemotherapy with sorafenib is the only therapeutic option for the patients with extrahepatic spread (EHS) of hepatocellular carcinoma (HCC). We previously developed a scoring system to predict prognosis for the patients with EHS before sorafenib approval. The score depends on uncontrollable intrahepatic lesions, extent of vascular invasion, and performance status (Cancer 2011). In this study, we evaluate the prognosis of HCC with EHS treated by sorafenib by using this scoring system.**Methods:** We enrolled 353 HCC cases diagnosed as EHS from 1993 to 2008, who have not received sorafenib as control group (C) and 47 cases with EHS from 2009 to 2015, who have been treated by sorafenib (S). The survival rate after the time of initial EHS diagnosis was calculated by the Kaplan–Meier method. We stratified them by the scoring system and liver function and compared the prognosis between S and C group by Log-rank test.**Results:** The survival rates at 1, 2, 3 years were 78.4, 51.8 and 34.8 % (S) and 38.9, 16.1 and 6.0 % (C). The MST was 742 days (S) and 248 days (C). The MST of Child A patients with score 0 was 1035 days (S: n = 19) vs 582 days (C: n = 46) (P = 0.028). The MST of Child A patients with score 1 was 709 days (S: n = 19) vs 301 days (C: n = 145) (P = 0.017).**Conclusion:** The prognosis of the sorafenib-treated patients with EHS has markedly improved, when they have good performance status and no uncontrollable intrahepatic lesions.

P-0660

MELD score predicts outcome of patients with HCC treated with Sorafenib**Andrea casadei gardini¹, Giorgia Marisi¹, Giovanni Luca Frassinetti¹**¹IRST-IRCCS, Meldola, Italy

The MELD score was initially created to predict survival in patients with complications of portal hypertension undergoing elective placement of transjugular intrahepatic portosystemic shunts (TIPS). The model was subsequently validated as a predictor of survival in several independent cohorts of patients with varying levels of liver disease severity but not in patients treated with sorafenib.

Methods: Our study included a training cohort of 67 HCC patients and a validation cohort of 61 patients receiving sorafenib. MELD score was measured before starting sorafenib.**Results:** An optimal cutoff point for the MELD score of 12 stratified the patients with HCC into high (greater 12) and low MELD score (lower 12) groups in the training cohort. In the training cohort, at univariate analysis, patients with meld score greater 12 had a lower median PFS (1.8 vs. 3.3 months, p = 0.004) and OS (6.9 vs. 8.7 months, p = 0.235) than patients with MELD score lower 12. These data were confirmed in the validation set in which patients with meld score greater 12 had a lower median PFS (1.4 vs. 5.6 months, p = 0.00001) and OS (5.0 vs. 13.9 months, p = 0.00001) than patients with MELD score lower 12. The multivariate analysis confirmed MELD score as the only independent prognostic factors in terms of PFS and OS.**Conclusions:** MELD score was a powerful prognostic indicator of poor outcome in patients with advanced HCC treated with sorafenib.

P-0661

NLR is predictive factor for survival in patients with advanced HCC treated by Sorafenib**Shuntaro Obi, Shinpei Sato, Takahisa Sato, Toshihiro Kawai, Takafumi Sugimoto, Miho Kanda**

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Background: Inflammation plays a critical role in the development and progression of various cancers [1]. Inflammation-related markers,

such as neutrophil to lymphocyte ratio (NLR), have been linked to clinical outcomes in patients with various malignancies. Sorafenib is now considered the first-line treatment for patients with advanced stage hepatocellular carcinoma (HCC) with good liver function worldwide. However, predictive factor of Sorafenib is not clear.

Aim: The aim of this study was to investigate the prognostic significance of the blood neutrophil-to-lymphocyte ratio (NLR) in patients with advanced HCC treated by Sorafenib.

Method: A total of one hundred nineteen patients with advanced HCC, not eligible for locoregional therapy, treated with Sorafenib were enrolled. The overall survival (OS) of patients with high NLR (>5) was compared with patients with low NLR. Clinical prognostic factors such as liver function, tumoral characteristics, and NLR were analyzed.

Result: Median OS was 8.9 months. The mean NLR at baseline was 2.76 (range 0.96–9.38). The median OS of patients with a high NLR was 5.8 months compared with 9.0 months for patients with a low NLR ($P = 0.018$). BCLC stage B, AFP-L3>36 %, Portal vein invasion, and NLR>5 were all predictors of poorer overall survival. Multivariate analysis showed that only NLR>5 was independent predictors of poorer overall survival.

Conclusion: High baseline NLR (NLR>5) was associated with worse OS for patients with advanced HCC treated with Sorafenib.

Reference: 1. Mantovani A, Allavena P, Sica A, Balkwill F. Cancer-related inflammation. *Nature*. 2008.

P-0662

Molecular target drug (sorafenib) might cause the activation of Kupffer cell

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Background: We have already reported that molecular target drug Sorafenib (SF) might lead apoptosis by increasing serum TNF-alpha and decreasing soluble Fas in patients with advanced hepatocellular carcinoma. It was reported that the length of microbubble (MB) collapse in liver parenchyma brought by repeated strong pulses in the post-vascular phase (Kupffer phase) of Sonazoid-enhanced ultrasonography (CE-US) reflects the function of Kupffer cell.

Aim: The aim of this study was to clarify the influence of the MB collapse by treatment of SF in liver cirrhosis patients (LC) with advanced hepatocellular carcinoma (aHCC).

Patients/method: Fifteen adult Japanese patients who had LC and aHCC were treated with SF. SF was administered at 400 mg/day for 4 weeks. Blood samples were collected before and after treatment. Serum levels of TNF-alpha and soluble Fas (sFas) were also evaluated. Flow cytometry was used to assess cytoplasmic IFN-gamma expression and other parameters for peripheral blood CD4+ T cells, and the percentage of IFN-gamma+, IL4- (Th1), IFN-gamma-, and IL4+ (Th2) was calculated. The CE-US was also underwent before and after treatment.

Result: There was the tendency of increase in serum levels of TNF-alpha and the Th2 cells after treatment. In the CE-US, the length of the collapsed MB was significantly decreased after treatment.

Furthermore, there are positive correlation between the length of the MB collapse and the Th2 cells.

Conclusions: SF might increase serum levels of TNF-alpha and decrease the MB collapse by inducing of activation of Kupffer cells in patients with LC and aHCC.

P-0663

RT combined with TACE in advanced HCC patient having hepatic vein invasion

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Background: Patients with advanced hepatocellular carcinoma (HCC) having hepatic vein invasion have a poor prognosis. We report four cases of radiation therapy (RT) combined with transarterial chemoembolization (TACE) in patients with advanced HCC having hepatic vein invasion.

Methods: To identify the feeding arteries of the tumor and hepatic vein invasion, we performed three-dimensional CT angiography using CT hepatic arteriography (CTHA) before TACE. Next, we selectively inserted a microballoon catheter into the feeding arteries. Subsequently, we injected a mixture of lipiodol and a fine-powder formulation of CDDP (IA-call[®]) into the tumor through the feeding branch. After, gelatin sponge particles were injected. We started RT (total dose of 50 Gy) aiming for the tip of the hepatic vein invasion within 1 month after TACE.

Results: All four cases had hepatic vein invasion extending up to the inferior vena cava. We found no serious adverse events during the treatment period. This treatment prevented the progression of hepatic vein invasion in all cases during the follow-up (median follow-up period: 4.5 months).

Conclusion: RT combined with TACE may be a therapeutic option for patients with advanced HCC having hepatic vein invasion.

P-0664

Stereotactic body radiation therapy for small hepatocellular carcinoma as a local salvage treatment

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Background: The aim of this study was to evaluate the efficacy and toxicity of stereotactic body radiation therapy (SBRT) as a local

salvage treatment for small, recurrent hepatocellular carcinoma (HCC).

Methods: Between March 2011 and February 2014, 44 patients with recurrent HCC (diameter <3 cm) treated by SBRT were reviewed in our institution. Prescribed SBRT doses were up to 60 gray (Gy) in 3 or 4 fractions. The tumor response was determined using dynamic computed tomography or magnetic resonance imaging, which was performed 3 months after completion of SBRT.

Results: Median size of tumors was 1.5 cm (range 1–3 cm). Median follow-up was 20.5 months (range 2–50 months). Thirty seven patients had Child-Turcotte-Pugh (CTP) class A disease, six patients had CTP class B disease, and one patient had CTP class C disease. The 1- and 2-year local control rate was 96.6 and 91.5 %, respectively. The 1-year overall survival rate was 75.3 %, and the progression-free survival rate was 76.5 %. Grade ≥ 2 hepatic toxicities were observed in 8 (18.2 %) patients. Grade ≥ 2 hepatic toxicities were more common in CTP class B or C disease than A disease (83.3 vs 7.9 %, $p < 0.01$). A high radiation dose was found to be independently not related to Grade ≥ 2 hepatic toxicity ($P = 0.088$).

Conclusions: This SBRT was effective in local control of small, recurrent HCC as salvage treatment.

P-0665

Clinical yield of radioembolization in hepatocellular carcinoma?

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Background and aim: Hepatocellular carcinoma (HCC) is one of the most common cancer in the world. Mortality can be decrease with early diagnosis and curative treatment modalities. We aimed to evaluate the clinical impact of RE in HCC patients.

Material and method: 59 unrespectable HCC patients were enrolled in this study. RE was performed in 28 of them (group 1) and 31 of these patients were followed-up in the natural course (group 2). All patients were classified according to the Barcelona clinic liver cancer (BCLC) staging classification. RE and natural course was compared with each other.

Results: Demographic features of patients were similar in two group. Mean age in RE group and natural course group were 60.3 ± 12.06 and 58.8 ± 10.7 years, respectively. In all patients were cirrhotic in group 1 (89.3 % Child A and 10.7 % B) and Barcelona classification were as follows: 60.7 % stage B and 39.3 % stage C. Multifocal involvement was detected in 57 % of patients. Nine pts of group 1 had previous TACE or sorafenib. In group 2, 83.9 % of patients were cirrhotic (22.6 % Child A, 38.7 % B, and % 22.6 C) and Barcelona classifications were as follows: 9.7 % stage B, 51.6 % stage C and 38.7 % stage D. Mortality rates were 82 % and 100 % in group 1 and 2, respectively. The mean survival periods were 480 days (95 % CI 344–615 days) and 247 days (95 % CI 160–334 days) in group 1 and 2, respectively (Log rank analysis, $P = 0.009$).

Conclusion: Mean survival time was higher in RE group as comparing with the natural course. However, previous treatment in RE patients may affect the survival. RE may be useful in earlier stage of HCC.

P-0666

Combined resection plus radiofrequency ablation versus transarterial embolization for BCLC-B HCC

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Background/aim: The aim of this study was to compare the outcomes of combined resection plus intraoperative radiofrequency ablation (RFA) and transarterial embolization (TAE) for Barcelona Clinic Liver Cancer (BCLC) stage B hepatocellular carcinoma (HCC) in case-controlled patient groups using the propensity score.

Methods: Between 2009 and 2014, 179 patients with multifocal HCC treated with resection plus intraoperative RFA ($n = 26$) or TAE ($n = 153$) were retrospectively studied. All patients were classified as BCLC stage B with Child-Pugh class A. Analyses were performed over all participants as well as for propensity score-matched (1:3) patients to adjust for baseline differences. Cumulative overall survival (OS) was compared between the two groups using the Kaplan–Meier method, and independent predictors were identified by multivariate Cox's regression analysis.

Results: At baseline, patients receiving combined therapy had lower aspartate aminotransferase level ($P = 0.002$), higher platelet count ($P = 0.035$), and less multi-nodularity ($n > 3$) than the TAE group. Univariate and subsequent multivariate Cox's regression analysis showed that combined therapy [hazard ratio (HR), 0.31; 95 % confidence interval (CI), 0.12–0.78; $P = 0.013$], BCLC substage B2 (HR 1.82; 95 % CI 1.13–2.92; $P = 0.013$) and alpha-fetoprotein >400 ng/ml (HR 1.85; 95 % CI 1.12–3.05; $P = 0.016$) were independent factors associated with OS. Propensity score-based analysis showed estimated 1-, 3-, and 5-year survival rates of patients receiving combined therapy and TAE were 95.7 vs. 81.0 %, 84.7 vs. 54.4 %, and 74.1 vs. 31.1 %, respectively ($P = 0.017$).

Conclusion: Combined resection plus RFA provides a better long-term OS compared with RFA in patients with BCLC stage B HCC.

P-0667

The effect of additional TACE after combined chemoradiation for locally advanced HCC with PVT

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Combined chemoradiation therapy (CCRT) followed by intraarterial chemotherapy (IAC) has been reported to be beneficial for locally advanced hepatocellular carcinoma (HCC) with portal vein thrombosis (PVT). However, hepatic arterial chemoembolization (TACE) was generally not accepted for this situation. The aim of this study is to evaluate the role of adding TACE instead of IAC after CCRT. Thirty-seven patients were treated by CCRT as the initial treatment for HCC with PVT. IAC through hepatic arterial chemoport and intermittent TACE (Group A) followed in 18 patients and only IAC were repeated in 19 patients (Group B). For CCRT, infusion of 5-FU via chemoport was given during the first and last 5 days of external radiation therapy (RTx) for 5 weeks. For IAC, infusion of cisplatin with 5-FU was repeated monthly. Mean age was 55 year; male to female was 32–5. Underlying liver diseases were hepatitis B, hepatitis C and non-B/C in 26, 2 and 10 patients, respectively. Mean follow-up duration was 14 months (3–65 months). The objective response (CR+PR) rates in tumor size after 1 month after CCRT were 45.9 %. The mean level of AFP after CCRT were decreased in 81 % of patients. During follow up, complete response was noted in 3 patients. One patient underwent resection for cure. Median overall survival duration was 12.4 months. Overall survival of group A was significantly better than that of group B. Adding intermittent transarterial chemoembolization contributed survival gain following combined chemoradiation therapy for advanced hepatocellular carcinoma with portal vein thrombosis.

P-0668

TACE followed by sorafenib improves progression free survival in patients with advanced HCC

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Objectives: To evaluate the efficacy and safety of the combination therapy with sorafenib and transarterial chemoembolization (TACE) as a treatment for advanced hepatocellular carcinoma (HCC).

Patients and methods: In this retrospective study we enrolled 95 advanced HCC patients treated with TACE between June 2009 and December 2012 in our institution. Of the 95 patients, 24 patients were treated with TACE followed by sorafenib (S-TACE group) and the 71 patients were treated with TACE alone (TACE alone group). Sorafenib was administered within 14 days after TACE. We compared the progression free survival (PFS) between the two groups and analyzed the predictive factors which affected on PFS.

Results: The median age was 72.2 years and 74 patients were male (77.9 %). Although the median tumor size was similar between the two groups, the mean numbers of tumors was significantly higher in S-TACE group: 16 vs. 8 ($P = 0.04$). The history of prior treatments was also significantly higher in S-TACE group. Other data was not significantly different between the two groups. There were no severe side effects between S-TACE group and TACE alone group. The mean PFS was significantly longer in S-TACE group: 176 days vs. 107 days ($P = 0.02$). Adjusting for significant factors in univariate analysis, multivariate analysis indicated that administration of sorafenib (OR: 0.38, $P = 0.01$), tumor size (OR: 1.12, $P = 0.01$) and ALT (HR: 1.04, $P = 0.01$) as independent factors which affected on PFS.

Conclusion: The combination therapy of sorafenib and TACE significantly improved PFS in patients with advanced HCC.

P-0669

RFA is comparable to RFA and TACE among patients with intermediate stage hepatocellular carcinoma

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Introduction: Hepatocellular carcinoma (HCC) ranks as the fourth most common cancer in the Philippines. Most of the HCCs found in the Philippines are at intermediate stage. Using Barcelona Clinic Liver Cancer (BCLC) staging, transarterial chemoembolization (TACE) is the most common recommended therapy for intermediate staged HCC. Recently, radiofrequency ablation (RFA) has been reported to be safe and effective as a first-line treatment for a single HCC patients diagnosed at intermediate stage. This study is to compare the survival of patients with intermediate sized HCC treated with TACE and RFA or RFA.

Methodology: Patients who were diagnosed with intermediate sized HCCs were grouped according to intended therapy: group A: RFA and TACE and group B: RFA. Data on demographics, clinical and laboratory parameters were recorded.

Results: The mean age was 63.37 years; mostly male (89.29 %) and Hepatitis B (60.71 %) as the leading cause of HCC. Mean main tumor size (cm) was 7.24 for group A and 6.42 for group B ($P = 0.364$). The overall survival ($P = 0.079$) or major complication rates ($p > 0.999$) between the two groups are not significant. The respective cumulative survival rates at 1, 3, 5, and 8 years were 97.6, 86.7, 74.5, and 60.0 % for group B and 93.4, 75.4, 63.1, and 51.1 % for group A. The median time to progression for group B and group A were 27.0 ± 3.8 (95 % CI 19.6–34.4) and 18.0 ± 2.9 (95 % CI 12.2–23.8) months, respectively.

Conclusion: The results show that TACE with RFA is comparable with RFA in terms of survivability and development of major complications.

P-0670

Frequency of surgical site infection after RFA without maximal sterile barrier precautions

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Introduction: Radiofrequency ablation (RFA) is widely performed in the treatment of hepatocellular carcinoma (HCC) and metastatic liver tumor. A guideline recommend use of maximal sterile barrier precautions (MSBP) during interventional radiology procedures. However, it is not clear that MSBP is necessary in RFA. RFA is not an angiographic procedure or nothing is implanted in RFA. We have performed RFA without MSBP. We investigated frequency of surgical site infection (SSI) after RFA for liver tumors without MSBP.

Materials and methods: From January 2014 to August 2015, 906 RFA treatments were performed for liver tumors at Juntendo University Hospital (Tokyo, Japan). 723 RFA treatments were performed for HCC, 183 RFA treatments were performed for metastatic liver tumor. All patients underwent RFA treatments without MSBP. We retrospectively reviewed database and investigated frequency of SSI and potential risk factor of liver abscess.

Results: SSI developed in 3 (0.3 %) of the 906 treatments. Subcutaneous abscess occurred in 1 treatment, liver abscess occurred in 2 treatments. 1 patient with liver abscess also developed lung abscess. Tumor diameter was greater than 30 mm in all cases with SSI. Liver abscess developed in 1 case of 2 treatments with enterobiliary reflux. Enterobiliary reflux and tumor diameter greater than 30 mm might be risk factors of SSI after RFA.

Conclusion: The incidence of SSI in our study (0.3 %) may be comparable to previous reports. MSBP may be unnecessary in RFA.

P-0671

Glisson's capsule injury after radiofrequency ablation in patients with hepatocellular carcinoma

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Aims: To investigate whether Glisson's capsule injury in radiofrequency ablation (RFA) affects the prognosis of patients with primary hepatocellular carcinoma (HCC).

Methods: We reviewed our database retrospectively and detected 182 primary HCC patients who were treated with RFA during April 2004 to December 2012. We defined (1) intrahepatic bile duct dilatation (affecting two or more subsegments), (2) hepatic arteriportal (AP) shunt (did not disappear or needed to be treated) or (3) hepatic infarction as Glisson's capsule injury. The relation between the incidence of Glisson's capsule injury and survival was analysed.

Results: The median age of the patients (male 121, female 61) was 74 years. The most frequent etiology was hepatitis C virus (75.2 %), followed by hepatitis B virus (9.3 %). 157 patients were Child-Pugh A and 25 patients were Child-Pugh B and C. Among these 182 patients, 15 patients showed Glisson's capsule injury (intrahepatic bile duct dilatation, n = 7; AP shunt, n = 9; hepatic infarction, n = 2: there is some overlapping). The median overall survival (OS) were 52.3 months (95 % CI 33.4–71.1) and 100.2 months (95 % CI 68.5–131.8) in patients with and without Glisson's capsule injury, respectively. There was significant difference between groups (P = 0.023). Multivariate analysis showed that Glisson's capsule injury (HR 2.12, P = 0.039) was an independent factor associated with OS.

Conclusions: Glisson's capsule injury in RFA may affect the prognosis in patients with hepatocellular carcinoma.

P-0672

Hepatic infarction after percutaneous radiofrequency ablation for liver tumors

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Background: Radiofrequency ablation (RFA) is widely accepted as an effective treatment for liver tumors. Although hepatic infarction

related to RFA varies in terms of severity, massive infarction could lead to fatal outcome. The aim of this study is to clarify the clinical courses of hepatic infarction after RFA.

Methods: We performed 8,118 RFA treatments for patients with hepatocellular carcinoma from February 1999 and December 2013. We identified cases with hepatic infarction by searching our prospectively designed database. We enrolled patients with severe hepatic infarction defined as a peak value of aspartate transaminase (AST) >500 IU/L after RFA. We retrieved clinical characteristics of the cases by chart review. We also analyzed liver function at 6 months after the hepatic infarction and investigated its association with the long-term survival.

Results: Severe hepatic infarctions occurred in 44 out of 8118 treatments (0.54 %), with one case of mortality within 30 days. Median peak value of AST was 835 IU/L (interquartile range 690–1186). Child-Pugh score (CPS) significantly increased 6 months after hepatic infarction (p < 0.001). Peak value of AST was significantly associated with increase in CPS at 6 months. The 5-year survival rate after hepatic infarction was 46.4 %, and the worsened liver function at 6 months was strongly associated with poor prognosis (68.2 % vs. 22.2 % at 5 years, p < 0.001).

Conclusions: Although severe hepatic infarctions related to RFA was rare, it can cause the deterioration of liver function and may affect the long-term survival.

P-0673

Terminal stage of hepatitis B related hepatocellular carcinoma is unsuitable for treatment

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Background: HCC is the third most common cause of cancer death worldwide. In Bangladesh hepatitis B is the most common cause of HCC accounting more than 61 % of cases and HBsAg positivity in the healthy population is 5.4 %. The outcome of the disease is related to the stage of presentation.

Objectives: Find out the BCLC staging in hepatitis B related HCC. **Methods:** This study was carried out in the Department of Hepatology, BSMMU, Dhaka and approved by IRB. The diagnosis of HCC was confirmed by pathological examination or AFP elevation (400 ng/ml) combined imaging (CT/MRI) after exclusion of hepatitis C virus infection and significant alcohol intake. All patients were HBsAg positive done by ELISA test. BCLC staging ranging from stage 0 to stage D.

Results: A total 44 patients were included in this study. Among them, 91 % were male. The mean age was 48.2 years with range from 23 to 80. Underlying Cirrhosis was seen in 79.5 % cases. Diagnostic level of serum AFP was seen in only 64 % with significant difference between cirrhosis HCC patients compared with non-cirrhosis HCC patients (77 vs. 33 %; P = 0.029). Biopsy was the best method to

diagnose HCC. Most cases (68 %) presented at terminal stage (BCLC Stage D).

Conclusions: The prevalence of terminal stage HCC makes most of the detectable lesions unsuitable for curative treatment. However, universal hepatitis B vaccination program may become the most effective preventive measure to control this disease in Bangladesh.

P-0674

Hepatocellular carcinoma received palliative treatments with statin

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Introduction: Worldwide, most hepatocellular carcinoma patients do not receive curative treatments for their disease. Alternative treatments for most HCC patients include palliative-intention medical modalities. The aim of our study was evaluate the effects of statin use in HCC patients receiving palliative treatment.

Materials and methods: The National Health Insurance claims database and cancer registry databases in Taiwan were linked for the analysis. Inclusion criteria were having HBV carrier-related HCC or not, being aged more 20 years, and having undergone TACE, radiotherapy, or chemotherapy as palliative treatment. Exclusion criteria were having been diagnosed with cancer before the HCC was confirmed, having undergone surgery, liver transplantation, radiofrequency ablation, or percutaneous ethanol injection as a curative-intent treatment, one's gender being unknown, having HCC diagnosed before HBV, and being younger than 20 years. The total number of enrolled hepatocellular carcinoma patients was 20,200 persons.

Results: HCC patients who received palliative treatment with statin use exhibited decreased HCC-specific deaths in all stages compared to those who received palliative treatment without statin use ($p = 0.0001$, $p = 0.0002$, $p = 0.0012$, and $p = 0.0002$, and relative risks of 0.763, 0.775, 0.839, and 0.718, respectively, for stages I–IV).

Conclusions: Palliative treatments are still important for HCC patients, and multiple therapeutic methods with statin use resulted in a lowest risk of death. Statins prolonged the survival of patients with advanced HCC who received palliative treatment, suggesting its value as an adjuvant treatment.

P-0675

Effect of intramuscular adipose tissue content on prognosis in patients undergoing HCC resection

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Background: It has recently been reported that myosteatosis, the infiltration of fat in skeletal muscle, is associated with insulin resistance and type 2 diabetes mellitus. The present study investigated the effect of skeletal muscle fat accumulation on short- and long-term outcomes following partial hepatectomy for hepatocellular carcinoma (HCC), and aimed to identify prognostic factors.

Methods: The records of 141 HCC patients who underwent hepatectomy were retrospectively reviewed. Clinicopathological and outcome data from 71 patients with high intramuscular adipose tissue content (IMAC) were compared with those from 70 patients with low IMAC.

Results: The 5-year overall survival rate was 46 % among patients with high IMAC and 75 % among those with low IMAC. The 5-year disease-free survival rates in these groups were 18 and 38 %, respectively. Multivariate analysis revealed that high IMAC was predictive of an unfavorable prognosis. High IMAC was significantly correlated with liver dysfunction, higher intraoperative blood loss, the need for blood transfusion, and comorbid diabetes mellitus.

Conclusions: Greater fat accumulation in skeletal muscle was predictive of worse overall survival after partial hepatectomy in patients with HCC, even with adjustment for other known predictors. The identification of patients with greater skeletal muscle fat accumulation before hepatectomy could permit early preventive strategies to maintain muscle quality, and thus improve prognosis and patient selection for hepatectomy.

P-0676

EUS-guided ethanol injection for hepatocellular carcinoma in S1 region

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Percutaneous ultrasound-guided radiofrequency ablation (RFA) for the treatment of hepatocellular carcinoma is common therapy worldwide, however RFA for HCC in S1 region is sometimes still challenging. We performed EUS-guided ethanol injection for treatment in S1 HCC. The patient was 72 years old female with LC (child A, etiology unknown). She was treated RFA for HCC in S4 region 4 months before the S1 recurrence developed. The size of S1 tumor was 20 × 13 mm and stage I (T1N0M0) according to UICC staging system. We performed EUS-guided ethanol injection for 3 times in a week and total injection volume of ethanol was 16 ml. No complication of this method was observed. After the treatment, we judged treatment effect (TE) was sufficient, but margin was insufficient (TE4b). Local recurrence in S1 region was observed 9 months later accompanied with recurrent HCC in S5 region, so we performed CT guided RFA for the local recurrence. After the treatment, no recurrence has been observed for 2 years. In this case, total volume of ethanol may be insufficient and injection to marginal area of tumor was difficult because of esophageal varices, which may be cause of local recurrence. EUS-guided ethanol injection might be a treatment option for S1 HCC, however further studies of larger numbers of patients will be needed to confirm the reliability of this method.

P-0677

ADI-PEG-20 as salvage therapy in a patient with advanced hepatocellular carcinoma

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Background: ADI-PEG-20 is arginine deiminase (ADI) formulated with polyethylene glycol and is known to inhibit the carcinoma growth. ADI-PEG-20 catabolize circulating arginine to yield citrulline and ammonia. It showed more effective to control hepatocellular carcinoma (HCC) in the phase II trial. We shared a case on compassionate use.

Case report: A 79-year-old male was well until HCC in left lobe liver and MALToma were found by health evaluation on Feb 2012. After surgery and systemic treatment, multiple recurrent HCCs were detected on Jun 2012. He received TACE and took target agents. However, the disease continued to deteriorate and worse. AFP was 21,405 ng/ml on Jan 2013. He started to ADI-PEG-20 injection weekly from Dec 28, 2012. Total 52 doses were prescribed. Each treatment dose of 160 IU/m² (18 mg/m²), with intramuscular way. Serum arginine decreased from 60.8 to 9.8 mM/L and citrulline increase from 12.9 to 150 mM/L after half year treatment, then return to initial level. During therapy, small LN was detected on neck. The recurrent lymphoma was detected at neck. The lymphoma was remission after CHOP treatment. Six months later, CT of abdomen revealed shrinkage of liver tumor, and AFP decreased to 1.08 ng/ml. He was still very well with stable disease on Aug 2015.

On conclusion, ADI-PEG-20 can suppress the growth of HCC. In some case, ADI-20 might have dramatic effect to kill cancer cell, especially with adding chemotoxic agent. From this patient's experience, combination therapy with ADI-PEG-20 might be more reasonable regimen for HCC therapy.

P-0678

Prognostic analyses of progressive disease patients with advanced Hepatoma treated with sorafenib

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Purpose: We examined progression pattern, treatment, and the prognosis about PD patients treated with sorafenib.

Methods: PD patients radiologically assessed were retrospectively examined. Overall survival (OS), time to progression (TTP), post-progression survival (PPS), progression pattern [(1) intrahepatic increase in tumor size, (2) extrahepatic increase in tumor size, (3) new intrahepatic lesion, (4) new extrahepatic lesion and (5) new intra/extrahepatic lesion], and prognostic factors were analyzed.

Result: 102 patients were radiologically assessed. PD was observed in 56 patients. The median OS, TTP, and PPS were 7, 1.9, and 5 months. The median OS by progression pattern was 7.5 months in (1), 15 in (2), 7.1 in (3), 6.9 in (4), and 2.6 in (5). Child-Pugh score of 5 point ($P = 0.004$) and invasion to intrahepatic major vessels ($P = 0.017$), at the beginning of sorafenib treatment were independent prognostic factors of OS by a Cox proportion hazard model. While, C-P A ($P = 0.036$), invasion to vessels ($P = 0.008$) and new intra/extrahepatic lesion ($P = 0.004$) were independent factors of PPS by a Cox model. Second line treatment for PD determined the good prognosis. Median PPS was 6.2 months in PD patients with second line treatment, while median PPS of 2.5 months without any treatment. C-P A of hepatic function was statistically observed in PD patients with second line treatment by Logistic-regression analysis ($P = 0.046$).

Conclusion: Hepatic function and progression pattern were important factors to determine the prognosis and second line treatment in PD patients.

P-0679

Prediction of response to transarterial chemotherapy with CDDP powder for hepatocellular carcinoma

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Aim: We retrospectively investigated the relationship between the tumor response and serial changes in α -fetoprotein (AFP) and des- γ -carboxy prothrombin (DCP) during hepatic arterial infusion of cisplatin powder formulation (CDDP powder) in patients with advanced HCC.

Patients and methods: Seventy-six advanced HCC patients were analyzed. All HCC patients received high-concentration cisplatin (1.43 mg/ml) via the hepatic artery at a dose of 65 mg/m². AFP and DCP were measured at baseline and 4–8 weeks after treatment, and the antitumor responses were evaluated according to the RECIST criteria after one or two courses of treatment. The patients were classified into two groups; a decreased group and a non-decreased group, based on the change in the serum levels of AFP and DCP at 4–8 weeks compared to baseline.

Results: The response to treatment of the decreased group ($n = 16$) and non-decreased group ($n = 60$) was CR/PR/SD/PD in 4/4/5/3 and 1/11/8/40 patients, respectively. The response rate and disease control rate of the decreased group were significantly higher than those of the non-decreased group ($P = 0.016$ and $P < 0.001$, respectively). The median survival time of the decreased/non-decreased groups were 25.9/10.6 months, respectively. The cumulative survival rates for the decreased group were significantly higher than those of the non-decreased group ($P = 0.042$). In the multivariate analysis, vascular invasion and the decrease group were significant factors that affected the therapeutic efficacy.

Conclusion: A decrease in the levels of AFP and DCP after the first treatment with CDDP powder was a good predictor for the antitumor effect and prognosis.

P-0680

Predictors of refractory chemoembolization in patients with hepatocellular carcinoma

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Transarterial chemoembolization is the first line therapy for BCLC intermediate stage HCC patients. However, still disease progressed after series embolization. In this study, we attempt to find out the predictors of TACE failure. Between January 2010 and May 2011, 474 fresh diagnosed HCC patients were treated with TACE in Chang Gung Memorial Hospital, Linkou branch. Patients without regular follow-up, with index TACE for tumor rupture or done in other

hospitals, or with combined therapy were excluded. Among 299 patients recruited, median age was 63.1 years old, 72.6 % were male, 89 % etiology were HBV/HCV, 84.6 % were cirrhotic, 88.8 % were Child-Pugh score classification A+B7, 55.9 % within BCLC stage B, 64.9 % HCC number <3, median size of the largest HCC was 3.5 cm. 47.8 % meet up-to-seven and the median follow-up duration was 31.9 (14.3–47.8) months. By comparison with the 18 patients defined as TACE failure to those non-failure, smoking (66.7 vs. 40.6 %, $P = 0.03$), BCLC stage C (77.8 vs. 42 %, $P = 0.011$), higher platelet count (131 vs. 108 K, $P = 0.028$), larger tumor size (7.4 vs. 3.5 cm, $P = 0.006$), up-to-seven (72.2 vs. 46.3 %, $P = 0.033$), macrovascular invasion (38.9 vs. 15.3 %, $P = 0.009$), and post-TACE fever (66.7 vs. 35.9 %, $P = 0.009$) were unfavorable factors. By multivariate analysis, beyond BCLC stage B3/B4/C (OR: 5.624, 95 % CI 1.580–20.01, $P = 0.008$), and post-TACE fever (OR: 3.364, 95 % CI 1.208–9.373, $P = 0.020$) were independent predictors of TACE failure. Our study found that advanced BCLC staging and post-TACE fever are independent predictive factors of TACE refractoriness. Patients encountered these factors may consider other treatment modality for the poor efficacy.

P-0681

Prognostic factors of the patients with TACE -refractory hepatocellular carcinoma

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Introduction: Transcatheter Arterial Chemo-Embolization (TACE) is a key therapy for intermediated stage hepatocellular carcinoma (HCC). When HCC progressed in spite of the treatment, the timing for switching to other treatment varies depending on the facility. In Japan, a concept of “TACE refractory” was proposed as the indication of sorafenib therapy, but TACE was often repeated with the expectation of its physical treatment effect. In this study, we investigated the prognosis of TACE refractory cases and examined the optimal treatment strategy.

Methods: We analyzed survival rate, patient’s background, background liver factor and tumor factor in 104 HCC patients who received TACE after “TACE refractory” by using Cox proportional hazard model and Kaplan–Meier method.

Results: The median age of the patients was 71 years old (12 HBV, 71 HCV) and the median survival time was 19.9 months. Multivariate analysis revealed that past treatment history (HR = 2.53, 95 % CI 1.29–5.33), tumor number more than 3 (HR = 2.73, 95 % CI 1.4–5.62), presence of vascular invasion (HR = 3.02, 95 % CI 1.08–7.54), low prothrombin (80 % or less, HR = 4.57, 95 % CI 2.28–9.04), high AFP (>40 ng/ml, HR = 2.63, 95 % CI 1.32–5.3), and high AFP-L3 (>10 %, HR = 2.75, 95 % CI 1.5–5.1) were the risk factors for survival. Twenty one cases survived more than 2 years after “TACE refractory” and the lack of emerging new nodules was selected as a factor for long survival (HR = 0.27, 95 % CI 0.08–0.91).

Conclusion: There were HCCs with good prognosis by repeating TACE after “TACE refractory” and we must decide next treatment based on the patients’ clinical characteristics.

P-0682

The FIB-4 index is a prognostic predictor for non-B non-C hepatocellular carcinoma

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Purpose: The aspartate aminotransferase to platelet ratio index (APRI) and FIB-4 index were developed as a noninvasive parameters for predicting liver fibrosis. This study aimed to validate the APRI and FIB-4 indexes in patients treated with curative hepatectomy for non-B non-C (NBNC) hepatocellular carcinoma (HCC).

Methods: Accumulated database comprising 399 patients who underwent hepatectomy was reviewed retrospectively. Analyses were performed to evaluate whether the APRI and FIB-4 indexes are predictors of liver cirrhosis and/or the prognosis in patients with NBNC-HCC.

Results: The APRI and FIB-4 indexes were significantly higher in the cirrhosis group than in the no cirrhosis group ($P = 0.001$ and $P < 0.001$, respectively). A time-dependent receiver operating characteristic curve analysis showed that the FIB-4 index was more accurate in predicting background liver cirrhosis than the APRI. According to a multivariate analysis, an FIB-4 index larger than 2.7 (hazard ratio: 2.51 and 2.21, 95 % CI 1.13–5.61 and 1.38–3.54, $P = 0.024$ and $P = 0.001$) remained significant independent predictors of disease-specific and recurrence-free survival, respectively.

Conclusions: The present findings showed that the FIB-4 index is a significant predictor of background liver cirrhosis and the prognosis after curative resection for NBNC-HCC.

P-0683

The FHCC-Pro: prognostic stratification index of filipino patients with HCC-A validation study

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Introduction: Prediction of survival of patients with hepatocellular carcinoma (HCC) is difficult and survival is dismal. Prognostic classification for patients with HCC is needed to determine proper treatment once diagnosed. The aim of this study is to develop a clinical prognostic scoring index predictive of survival for patients with HCC.

Methods: Patients with HCC from January 2003 to September 2014 were included. The following data were collected: FHCC-Pro score (DM: NO = 0, YES = 2; ALCOHOL: NO = 0, YES = 2; TUMOR SIZE: <3 cm = 0, >3 cm = 2; TUMOR#: SINGLE = 0, >2 = 2; AFP: <10 = 0, >10 = 2; CPC: A = 0, B = 1, C = 2). The prognosis scores were categorized GOOD (scored 0–4), INTERMEDIATE (scored 5–7) or POOR (8–12). Treatment interventions noted (RFA/TACE). Final end point measured using one-year survival from time of diagnosis until time of death (months).

Results: A total of 58 patients diagnosed with HCC were included. The 1-year survival rates according to class are: GOOD 94 %, INTERMEDIATE 60 %, and POOR 27 %. Overall survival rates for

those who received RFA and/or TACE was higher than those with supportive treatment alone (71 vs 29.7 %). Sub stratification of patients who received TACE had a higher survival rate than those who received RFA. (85.7 and 64.3 % respectively).

Conclusion: Presence of diabetes, significant alcohol consumption, tumor size >3 cm, multiple tumors, elevated AFP and Child-Pugh classification correlate with poor survival. A scoring system such as this model may be used to predict survival of patients with HCC.

P-0684

Role of VEGF as a predictor of survival in HCC patients receiving supportive treatment

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Background: In low-resource countries, the limited availability of palliative treatment, such as transarterial chemoembolization, may cause many intermediate or advanced stage HCC (hepatocellular carcinoma) patients to get only supportive treatment. The role of VEGF in predicting survival in HCC patients who are given supportive therapies has not been elucidated.

Methods: Serum VEGF levels at diagnosis were measured in sixty HCC patients given supportive treatment (age ranged between 23 and 78) from August 2014 to March 2015. Serum VEGF levels were measured using ELISA method. The cut-off value of VEGF was obtained using receiver operating characteristic (ROC) curve. The prognostic significance of VEGF cut-off and other parameters were analyzed using univariate and multivariate analysis. The correlation of VEGF and BCLC stage was analyzed using Spearman's correlation test.

Results: Serum VEGF level correlates significantly with BCLC stage ($p < 0.001$). Using ROC curve, the optimal cutoff for VEGF to predict mortality was 226.5 pg/mL. Overall median survival was 138 days. Although the six-month survival rate is better in the low serum VEGF group (61.4 %) compared with the high serum VEGF group (38.8 %), the difference was not statistically significant ($P = 0.105$). In multivariate analysis, BCLC stage was the only independent predictor of survival. In subgroup analysis, the survival of low and high serum VEGF groups according to BCLC stage were not statistically significant.

Conclusion: There was a correlation between serum VEGF level and BCLC stage, but VEGF level could not predict the outcome of HCC patients receiving supportive treatment.

Keywords: Hepatocellular carcinoma, VEGF, survival, Supportive treatment

P-0685

Predictive factors for local tumor progression after radiofrequency ablation for HCC

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Aim: The aim was to identify factors for achievement of a sufficient ablative margin in RFA for HCC and predictors for local tumor progression.

Methods: A total of 212 HCCs in 141 patients were treated by RFA. Enhanced CT were performed within 1 month after RFA. AM was categorized three grades. AM+, a 5 mm or larger AM round the entire tumor; AMzero, an AM round the tumor but a less than 5 mm in part; and AM–, incomplete AM around the tumor but no residual tumor.

Results: AM+, AMzero, and AM– were found in 122, 77, and 13 nodules, respectively. In comparison of the baseline characteristics between AM+ and nonAM+ nodules, significant differences were found in tumor size, subcapsular location and the combination of TACE. Multivariate analysis showed that a significant factor for the achievement of AM+ was the combination of TACE and subcapsular location. The cumulative local tumor progression rates 2.6, 6.4 and 8.2 % at 1, 2, and 3 years, respectively in 122 AM+ nodules were significantly lower than those 8.9, 29.1, and 37.0 % in 90 nonAM+ nodules. Univariate analysis showed that hypervascularity, tumor size, gross classification of HCC, contiguous vessels and AM grading were significant risk factors. A multivariate Cox proportional hazards model identified contiguous vessels and gross classification as an independent predictor for local tumor progression.

Conclusion: The presence of contiguous vessels and gross classification were more important predictors for local tumor progression than AM grading.

P-0686

ADOPT-LC score: a novel score for in-hospital mortality of surgical procedure in cirrhotic patients

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Background: The majority of previous studies that evaluated risk factors for in-hospital mortality of cirrhotic patients following surgical procedures were limited by a small sample size.

Methods: Using a total of 12 million inpatients registered in a Japanese nationwide administrative database, we enrolled 2197 cirrhotic patients who underwent elective ($n = 1973$) or emergency ($n = 224$) surgery. We analyzed the risk factors for postoperative mortality and established a scoring system for predicting postoperative mortality in cirrhotic patients using a split-sample method with a training and a testing set.

Results: In-hospital mortality rates following elective or emergency surgery were 4.7 and 20.5 %, respectively. In multivariate analysis, patient age, Child-Pugh (CP) class, Charlson Comorbidity Index (CCI), and duration of anesthesia in elective surgery; and CP class and duration of anesthesia in emergency surgery were significantly associated with in-hospital mortality. Based on multivariate analysis in the training set ($n = 987$), the adequate operative treatment for liver cirrhosis (ADOPT-LC) score that used patient age, CP class,

CCI and duration of anesthesia to predict in-hospital mortality following elective surgery was developed. This scoring system was validated in the testing set ($n = 986$) and produced an area under the curve (AUC) of 0.881.

Conclusion: Patient age, CP class, CCI and duration of anesthesia were identified as important risk factors for predicting postoperative mortality in cirrhotic patients. The ADOPT-LC score effectively predicts in-hospital mortality following elective surgery and may assist decisions regarding surgical procedures in cirrhotic patients based on a quantitative risk assessment.

P-0687

Preoperative platelet-to-lymphocyte ratio (PLR) predicts recurrence status after initial hepatectomy

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Introduction: The recurrence patterns after initial hepatectomy were a predictive factor for the patients with HCC and important for the choice of the later treatment policy. We analyzed the clinical usefulness of PLR score in patients with HCC who underwent initial radical hepatectomy.

Materials and methods: From January 2007 to December 2012, 271 patients with HCC initially underwent curative liver resection at our institution. All patients were classified into two groups: low-PLR (<150) group (15.5 %) and high-PLR (≥ 150) group (84.5 %). The patients were divided into a group with a recurrence beyond Milan criteria (MC) and one within MC and no recurrence according to the recurrence patterns after initial hepatectomy. We then analyzed the association between the recurrence patterns and the clinicopathological factors.

Results: 55 (20.2 %) patients had a recurrence beyond MC and 216 (79.8 %) patients the other recurrence pattern. The five-year survival rate in groups with the other recurrence pattern (79.7 %) was significantly higher than that in the group with the recurrence beyond MC (41.6 %, $p < 0.0001$). Univariate analysis revealed PIVKAI levels, surgical procedure, extent to liver resection, tumor size, number of tumors, clinical vascular invasion, MC before resection and PLR, then multivariate analysis revealed high-PLR (RR: 3.21, $P = 0.0028$) and beyond MC before resection (RR: 2.81, $P = 0.0092$) as independent predictive factors for recurrence of HCC. The high-PLR (≥ 150) group (15.5 %) was significantly associated with great frequent poor histological differentiation ($P = 0.014$).

Conclusion: Preoperative high-PLR is useful as predictive marker for the recurrence beyond MC after initial hepatectomy.

P-0688

Surveillance of viral hepatitis affects not only the detection of early-stage HCC but also survival

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Aim: This study aimed to reveal the benefits of surveillance of patients with viral hepatitis.

Method: Subjects were consecutive patients with viral infection who had been diagnosed with or treated for HCC at our hospital from 2004 to 2012. We retrospectively analyzed the history of the current illness in the hospital discharge summary. To minimize the lead-time bias, the corrected survival for patients with surveillance was calculated.

Results: Of 333 patients analyzed in this study, 107 (32.1 %) did not have surveillance and had low cumulative survival rates compared with those who had surveillance. The median corrected survival was 51.5 months in patients with surveillance, but the median observed survival of patients without surveillance was 31.4 months ($P = 0.011$). Multivariate analysis revealed that AFP ≤ 35 [odds ratio (OR), 2.054], Child-Pugh A (OR 2.488) and ideal follow-up (OR 4.539) were independent factors associated with the detection of early-stage HCC, and age (OR 0.939), tumor stage I/II (OR 6.918) and ideal follow-up (OR 3.213) were independent factors associated with receiving curative treatments.

Conclusions: Surveillance of patients with viral hepatitis independently increased the opportunity of detecting early-stage HCC and receiving curative treatments. Patients with surveillance had a better survival.

P-0689

Saffron-based crocin prevents liver cancer: in vivo, in vitro and in silico insights

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Hepatocellular carcinoma (HCC) is the second most common cause of cancer-related death worldwide. The prognosis of patients with HCC is usually poor; hence, a novel approach against HCC is essential for a better therapeutic outcome. Saffron and its active constituents were reported to have antioxidant, anti-inflammatory, and anti-tumor properties. The aim of this study was to investigate the chemopreventive action of crocin, one of the main active constituents of saffron, against diethylnitrosamine (DEN)-induced liver cancer in rats, and the possible mechanisms by which crocin exerts its anti-tumor effects. Findings reported herein demonstrated the anti-proliferative and pro-apoptotic properties of crocin when administrated in DEN-treated rats. Additionally, crocin exhibited anti-inflammatory properties where NF- κ B, among other inflammatory markers, was inhibited. In vitro analysis confirmed crocin's effect in HepG2 where cell cycle was arrested at G2, apoptosis was induced and inflammation was down regulated. Network analysis identified NF- κ B as a regulatory hub, and therefore, a candidate therapeutic drug target. Taken together, the presented results introduce crocin as a candidate chemopreventive agent against HCC.

P-0690

A cost of illness analysis of HCC in the setting of severe financial constraints

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Background: Hepatocellular Carcinoma (HCC) is associated with increased incidence and mortality and, thus, an escalating disease and financial burden. Patient level data on healthcare resource use and cost-of-illness analyses of HCC remain rather scarce. The objective of this study was to estimate costs per patient with HCC in Greece.

Methods: 123 patients with HCC followed in a dedicated clinic of a university hospital in Greece. Detailed resource use data were derived from the medical records. Data were recorded from the first encounter with the facility until loss of follow-up occurred. Calculations follow a third-party payer perspective, according to official tariffs, and costs are expressed in year 2015 Euros.

Results: 77 % were male with average age of 73.1 years and a median follow-up of 6 months. Cirrhosis was present at 83.7 %. Overall, the average cost/patient/month was 2751.6E.

Average total cost per patient with a complete follow-up was 10705.62E. Major cost drivers were hospitalizations (55.4 %), interventional procedures (28.3 %) and medications (10.6 %). Cost per month of follow up was significantly higher for patients with cirrhosis compared to non-cirrhotic (3745.2 vs. 2550.8 respectively, $p < 0.05$) and according to underlying disease were 3701.6E, 2898.4, 2967.6 and 1033.4 for patients with alcoholic steatohepatitis, HBV, HCV and NAFLD, respectively.

Conclusions: Albeit slightly lower than the estimates reported for other settings, HCC represents a major burden for the health care system, the patients and the society in Greece. Disease progression, as proxies in this analysis by the presence of cirrhosis, significantly increases the economic burden associated with the disease

P-0691

Alcoholism concomitant with viral hepatitis increases the prevalence of HCC in Taiwan

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Background and aims: Taiwan has a high prevalence of HBV infection, HCV infection, and HCC with increasing consumption of alcohol. We investigated the etiology of HCC among alcoholism and viral hepatitis.

Methods: We retrospectively collected 1506 HCC patients between 2012 and 2015 at the E-Da hospital. All patients were tested with HBsAg and anti-HCV. HCC was diagnosed on the basis of histological examination, or typical findings of multi-dynamic CT or MRI according to APASL HCC guidelines. The definite of alcoholism is drinking more than 20 g per day and over 5 years.

Results: Fifteen hundreds and six HCC patients were included. The rate was 37, 35, 5, and 23 % in patients with HBV infection, HCV infection, HBV/HCV infection, and non-HBV/HCV infection, respectively. Moreover, the rate was 80 and 20 % in HCC patients with alcoholism and non-alcoholism, respectively. For the 296 patients with alcoholism, the rate was 43, 28, 5, and 24 % in alcoholic patients with HBV infection, HCV infection, HBV/HCV infection, and alcoholism only, respectively. For 552 HBV patients, the rate was 77 and 23 % in patients with alcoholism and non-alcoholism, respectively. For 528 HCV patients, the rate was 84 and 16 % in patients with alcoholism and non-alcoholism, respectively.

Conclusions: Viral hepatitis results in about 80 % of HCC. However, the rate of alcoholism is increasing in HCC patients and alcoholism concomitant with viral hepatitis also increases the rate of HCC in Taiwan.

P-0692

Introduction of Taiwan liver cancer network

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Recent advances in genomics, proteomics, and increasing demands for biomarker validation studies have changed the landscape of cancer research. The establishment of tissue bank for translational research became very important. Hepatocellular carcinoma (HCC) is the number one of the ten leading cancer death in Taiwan, so Taiwan liver cancer network (TLCN) was organized by National Research Program for Genomic Medicine (NRPGM) in year 2005. The goal of TLCN was to provide high quality biosamples with reliable and sufficient clinical information for scientists in Taiwan to facilitate the liver cancer research and achieve break through in HCC treatment and prevention. TLCN coordinated five major medical centers in Taiwan to collect tumor tissues and blood biosamples of liver tumor patients along with comprehensive clinical and epidemiological data. The five medical centers are located in the northern (National Taiwan University Hospital and Chang Gung Memorial Hospital Linko Branch), central (Taichung Veteran General Hospital), and southern parts (Chang Gung Memorial Hospital Kaohsiung Branch and Kaohsiung Veteran General Hospital) of Taiwan. All participating centers must follow a common protocol to collect biosamples as well as the patients' clinical, pathological and epidemiological information. By Sep. 30, 2015, we have successfully recruited more than 7600 liver tumor patients with biosamples. So far, we already have 107 applications, and have sent out more than 41,000 biosamples. Our Applicants have published 44 papers with high impact factors. Taiwan Liver Cancer Network has successfully become the most important resource for the liver cancer research in Taiwan.

P-0693

Effect of type-2 diabetes mellitus on blood pressure in hepatocellular carcinoma**Chun Gao, Long Fang, Wei-Shuo Zhang**

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Background: Type-2 diabetes mellitus (DM) has been associated with hypertension and hepatocellular carcinoma (HCC) our study was designed to determine the effect of type-2 DM on the blood pressure in Chinese HCC patients.

Methods: A total of 375 patients treated at the China-Japan Friendship Hospital in the period January 2003 to April 2012 and with a hospital discharge diagnosis of HCC were studied. The demographic clinical biochemical and metabolic features/data were analyzed; and the multivariate logistic regression model was used to determine the effect of DM on the blood pressure in HCC.

Results: Of the total 375 patients 63 (16.8 %) were diagnosed with DM and 88 patients (23.5 %) had reported to have the past history of hypertension. The mean systolic blood pressure (SBP) was 130 mmHg and the mean diastolic blood pressure (DBP) was 79 mmHg. Univariate analysis showed that a statistical difference was found for the SBP (133 vs. 129 mmHg $P = 0.048$) not for the DBP and mean artery pressure (MAP). In the multivariate analysis after controlled by age hemoglobin and platelet count SBP was shown to be as an independent factor associated with HCC patients with type-2 DM (odds ratio 1.017, 95 % confidence interval 1.001–1.034, $P = 0.039$).

Conclusions: Type-2 DM affects the systolic blood pressure (SBP) of our Chinese HCC patients not the DBP and MAP although large-scale prospective cohort studies are required.

P-0694

Insulin treatment can accelerate the recurrence of hepatocellular carcinoma after surgery**Hayato Baba^{1,2}, Kazuto Shibuya², Koushi Matsui², Kazuhiro Tsukada²**

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Aim: Insulin treatment is a possible risk factor for hepatocellular carcinoma (HCC) in patients with diabetes mellitus (DM). However, the effect of insulin treatment on the prognosis after the surgical treatment of HCC is unclear. In the present study, we examined the effect of insulin treatment on the prognosis of HCC patients after hepatectomy.

Methods: We examined 124 HCC patients who underwent hepatectomy from 2004 to 2014 at our hospital and met the following criteria: (1) hepatectomy was the initial treatment for HCC and (2) surgery was curable. We focused on the treatment for DM after surgery, which was reviewed retrospectively from the medical records.

Results: Out of 124 patients, 54 (44 %) were diagnosed with DM. Relapse-free survival (RFS) and overall survival (OS) were not significantly different between the DM and non-DM groups. Out of 54 patients with DM, 15 (28 %) were treated with insulin. RFS was

significantly shorter in patients who received insulin treatment than in those who did not (median RFS 468 vs. 1376 days, respectively; log-rank $p = 0.0121$), but OS was not different between both groups. RFS remained significantly different even after adjustment for other factors such as sex, age, liver disease, Child–Pugh class, histopathology of background liver, and clinical stage (hazard ratio 4.30, $p = 0.0016$). On the other hand, RFS and OS were not significantly different between groups with low HbA1c (<7.5 %) and high HbA1c (≥ 7.5 %).

Conclusion: Insulin treatment can be a prognostic factor for recurrence after surgery in HCC patients.

P-0695

Effect of type-2 diabetes mellitus on platelet count in hepatocellular carcinoma**Long Fang, Wei-Shuo Zhang, Chun Gao**

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Aim: Our previous study showed that the international normalized ratio/INR level was affected by type-2 diabetes mellitus (DM) in hepatocellular carcinoma (HCC) patients, in addition, platelet plays important roles in the process of coagulation, this current study was designed to determine the effect of type-2 DM on the platelet count in Chinese HCC patients.

Methods: A total of 375 patients, treated at the China-Japan Friendship Hospital in the period January 2003 to April 2012, and with a hospital discharge diagnosis of HCC, were studied, according to the diagnostic criteria, inclusion and exclusion criteria. The demographic, clinical, biochemical and metabolic features/data were analyzed; and the multivariate logistic regression model was used to determine the effect of DM on the platelet count in HCC.

Results: Of the total 375 patients, 63 (16.8 %) were diagnosed with DM, the mean neutrophil was $4.13 \pm 2.49 \times 9/L$, the mean hemoglobin was 132.5 ± 23.8 g/L, the median/interquartile- range of platelet count was $130 (85–189) \times 9/L$ and the mean INR level was 1.20 ± 0.27 . A statistical difference was found by univariate analysis for the platelet count [$113/(64–157) \times 9/L$ vs. $139/(89–192) \times 9/L$, $P = 0.020$]. However, no statistical difference (odds ratio 0.999, 95 % CI 0.995–1.003, $P = 0.505$) was found by the multivariate analysis, after controlled by age, HBV infection, hemoglobin and INR.

Conclusions: HCC patients with type-2 DM have the decreased platelet count, compared with those HCC patients without DM, which was modified by other factors.

P-0696

38 cases of brain metastases from hepatocellular carcinoma**Takahisa Sato, Toshihiro Kawai, Takafumi Sugimoto, Miho Kanda, Shinpei Sato, Shuntaro Obi**

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Background: Brain metastasis is empirically recognized to be extremely poor prognosis, especially in advanced hepatocellular carcinoma with extrahepatic metastasis. However, a therapeutic options and prognosis of brain metastasis is not fully reported.

Patients: Between April 2001 and May 2015, 38 cases of brain metastases from hepatocellular carcinoma were diagnosed in our hospital. We examined prognosis, therapeutic options, and the risk of developing brain metastasis.

Results: For the 38 patients [mean age 64.0 ± 12.2 ; male/female = 30/8; HBV/HCV/HBV+HCV/NBNC = 14/17/1/6; Vp 0/1/2/3/4 = 21/0/4/9/4; Vv 0/1/2/3 = 0/0/0/11; stage 1/2/3/4a/4b = 0/0/1/1/36; lung/bone/lymph 35/3/3 (with overlaps); median size 24 mm; number of brain tumors 1/2/>3 = 28/5/5 patients], the median survival time was 33 days, with 37 deaths which were all due to cancer. 35 of 38 patients were diagnosed by central nervous system manifestation, and the other three patients were asymptomatic. All the patients were treated with dexamethasone and glycerin, and 13 patients were transferred to another hospital for additional treatment. Eight of those patients were treated with gamma knife, one patient was treated with cyber knife, three patients were underwent surgical removal of hematoma, and three patients were treated with radiation therapy (with overlaps).

Conclusion: 35 of 38 cases of brain metastases were accompanied by pulmonary metastasis, and the pulmonary metastasis was considered to be the strongest risk factor of brain metastasis. Four patients who received gamma knife and two patients who received radiation therapy achieved survival more than 6 months, the effectiveness of the gamma knife and radiation for brain metastasis of hepatocellular carcinoma was suggested.

P-0697

Failure to detect: late hepatocellular carcinoma (HCC) recurrence after liver transplantation (LT)

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Introduction: Current recommendations for post-transplant HCC surveillance consist of ultrasonography every 3–6 months in the first 2 years followed by annual ultrasonography along with alpha-feto-protein (AFP) every 6 months. However, it is controversial whether this is sufficient, especially in patients with poor prognostic factors. We present a case of late post-transplant HCC recurrence despite adequate surveillance 11 years after transplantation.

Methods and results: A 63-year-old male with chronic Hepatitis B cirrhosis and HCC underwent liver resection in 2002 and LT in 2004 following recurrence. Explant pathology confirmed HCC with venous invasion. He received post adjuvant oral Capecitabine for 5 years, Hepatitis B Immunoglobulin, Mycophenolate Mofetil, Lamivudine, and Tacrolimus. Surveillance with ultrasonography and AFP at 6-month intervals were negative over 10 years. In January 2015 he presented with fever of unknown origin. Initial CT scan showed abnormal attenuation in the left lobe and a 5.2×4.8 cm mass anterior to the liver. Subsequent scan in 2 months showed multi-foci disease (largest measuring $12 \times 18 \times 15$ cm) and enlargement of the extrahepatic mass to 6.1×5.6 cm. AFP rose from 39,032 to 82,810 $\mu\text{g/L}$. Biopsies of the liver and mass confirmed HCC.

Conclusion: Though most HCC recurrence occurs within 24 months of LT, late recurrence can occur especially in patients with poor prognostic factors including previous tumour recurrence, micro-

vascular invasion and HBV infection. In this case, screening intervals more vigilant than guideline recommendations failed to detect early HCC recurrence. This begs the question whether a different imaging modality would have made an impact in early tumor detection.

P-0698

Hepatic actinomycosis mimicking hepatocellular carcinoma

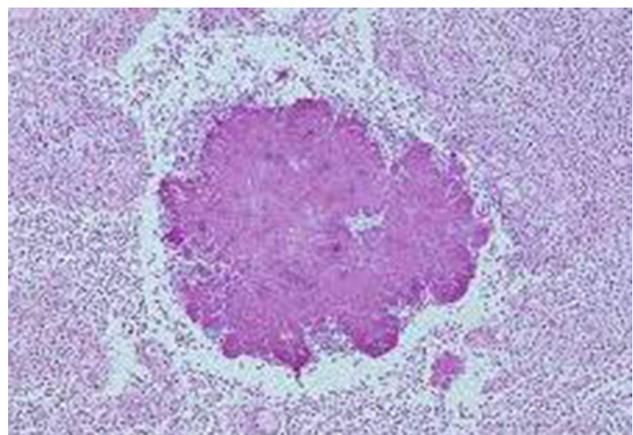
Dewa Pakshage Chula Kanishka Ananda Lal, Sivasooriya Sivaganesh

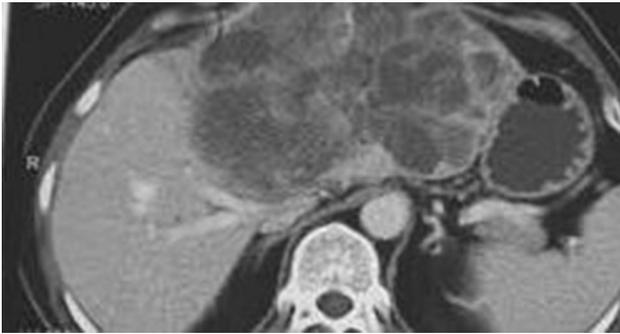
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Introduction: Actinomycosis is a rare bacterial infection caused by *Actinomyces Israeli* which is found as a commensal in gastrointestinal and female genital tract. Diagnosis of deep seated actinomycotic abscess is difficult as it mimics neoplasms in imaging. Antibiotic of choice is conventional penicillin and 4–6 weeks parenteral followed by 6 months oral treatment is recommended to prevent recurrence.

Case report: A 42 year old otherwise healthy person presented with abdominal pain and fever for 10 days duration. Examination revealed an acute abdomen and evidence of sepsis. Haematological and biochemical investigations were in favor of an acute inflammatory condition due to a pyogenic infection. Radiological evaluation was suspicious of a hepatic neoplasm or an abscess. Aspirate of the cystic areas of the lesion was negative for an infective etiology but biopsy of solid areas showed histopathological evidence of actinomycosis. Patient was started on benzyl penicillin and then converted co-amoxiclavate and clindamycin (based on Microbiology opinion) due to practical problems of long term parenteral penicillin therapy. Patient was treated intravenously for 2 weeks and discharged on oral antibiotics for 4 weeks. Repeat imaging was planned in 1 month, but patient lost to follow up.

Discussion and conclusion: Hepatic Actinomycosis should be considered an important differential diagnosis in patients with features of sepsis and evidence of hepatic neoplasm in imaging. Surgical interventions are not indicated if patient is not deteriorating but should be followed up with imaging to exclude concomitant neoplasm.





P-0699

Fibrolamellar HCC hepatectomy and postoperative acute vena cava syndrome and successful thrombectomy

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An 18-year-old female presented with recurrent epigastric pain and 4 kg weight loss. An abdominal computerised tomography scan showed a great hepatic tumor, dependent of the lateral segment of the left lobule 21 × 15.8 × 12.6 cm highly suggestive of fibrolamellar hepatocellular carcinoma (FHC). The lesion causes mass effect on the portal and left suprahepatic vein branches. Serum alpha-fetoprotein was not elevated and hepatitis B and C were negative. Left hepatectomy of the segments 2, 3, 4a and partial 4b, splenectomy and cholecystectomy was performed. After surgery and closing of the abdominal cavity, jugular plethora and face edema is observed; therefore, it was decided to perform an immediate contrasted tomography and angiography of the right jugular vein and superior vena cava (SVC) showing an extensive thrombus. Then, seven sequential aspirations of peripheral thrombus were performed, obtaining abundant fresh thrombotic material. Subsequently, angioplasty showed the luminal gain memory of the area occluded by the thrombus, conditioning partial drainage into right cardiac cavities. Through a venous catheter, positioned in the site of the thrombotic occlusion, administering a continuous infusion of alteplase for thrombolytic therapy. Control angiography in the right jugular vein and SVC, shows improvement of the venous flow, from the internal jugular vein to the junction with the SVC. Drainage into the left atrium showed practically normal flow. Medical management is continued with heparin. Histological diagnosis of fibrolamellar hepatocellular carcinoma was confirmed. The patient is disclosed with oral anticoagulation. After 6 months the patient is alive and free of disease.

P-0700

A case of liver tumors caused by lymphoproliferative disorder in Crohn's disease clinical course

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A 27-year-old man who was suffering with Crohn's disease (CD) for more than 10 years, was not achieved in remission despite of the administration with 5-aminosalicylic acid (5-ASA), immune modulator, and biologics. He came with a complaint of abdominal pain and fever, showed multiple liver tumors and wall thickening and narrowing of the descending colon on the abdominal enhanced CT. The liver tumors were enlarged comparing with previous abdominal enhanced CT retrospectively, percutaneous liver biopsy was required to rule out adenocarcinoma metastasis or inflammatory pseudo-tumor. After admission with the diagnosis of ileus, his condition was improved. However the pathologic diagnosis of the biopsy was diffuse large B-cell lymphoma with EB virus-positive caused in the iatrogenic immunodeficiency by immunosuppressant and biologics. Treated with the current standard chemotherapy for malignant lymphoma induced by the department of hematology in our university hospital, the size of the liver tumors on the following abdominal enhanced CT became significantly smaller than before. However the primary lesion of the lymphoma at the descending colon which was diagnosed the most severe inflamed section in his gut, was difficult to achieve clinical remission. After resection of the primary section, salvage therapy of the malignant lymphoma was started to him. In previous report, the development of lymphoproliferative disorder was not related with administration of immunosuppressant among Japanese CD patients, although the risk increase of lymphoma induced by immunomodulatory agents was already reported overseas. To establish the surveillance of the iatrogenic immunodeficiency lymphoproliferative disorder should be urgent project.

P-0701

A case of metachronous spontaneous regression of hepatocellular carcinoma

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Introduction: We herein describe a case of metachronous spontaneous tumor regression of hepatocellular carcinoma (HCC).

Case: A 78 year old man had been followed up for chronic hepatitis C, undergoing ultrasonography (US) every 6 months. In February 20xx, US revealed a 25 mm hypoechoic tumor in liver segment S5.

Contrast enhanced computed tomography confirmed a diagnosis of HCC. He was followed up without treatment because of depression. His AFP level was 9.2 ng/ml. In December 20xx, the tumor grew to 46 mm and then started shrinking. In May 20xx + 2, it decreased to 30 mm. In April 20xx + 3, a 17 mm tumor was detected in S4-8 by US, and recurrence of HCC in another liver segment was diagnosed. The tumor in S4-8 had grown to 30 mm by August 20xx + 3, and AFP was markedly increased to 500 or more. Although US performed in February 20xx + 4 revealed growth of the tumor to 42 mm, it started shrinking in May. In November 20xx + 4, the tumor measured 20 mm. As for tumor markers, AFP normalized. Regression of HCCs was uneventful.

Discussion: Spontaneous regression of HCC is assumed to result from the additive effects of ischemic factors in a lesion with an abundant blood flow, involvement of the immune system, depletion of growth factors, and so forth. In our case, it also appeared that spontaneous regression might have been caused by host factors.

Conclusion: We experienced a case showing metachronous spontaneous regression of HCC. This case raises the interesting possibilities of multiple, interacting causes of spontaneous regression.

P-0702

A case of the large hepatocellular carcinoma effective treatment with modified NEW FP method

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It was difficult treatment except surgical resection for the large hepatocellular carcinoma (HCC). Because these cases were inadequacy liver function and they were impossible for hepatectomy. NEW FP for large HCC treatment proposed Dr. Nagamatsu, and he reported NEW FP was highly effect. We reported our experienced case of marked effect NEW FP for the large HCC. A 62 year old woman had treatment for pneumonia, who was found a large HCC at right lobe of the liver unexpectedly CT scan examination. The large HCC was occupied segment 8 and including middle hepatic vein highly suspected. AFP 101.05 ng/ml (cut-off 10 ng/ml) and PIVKA-2 2177 mAU/ml (cut-off 37 mAU/ml) were elevated. The liver function was deterioration because ICG test 34 % (cut-off 5 %). We were going to focus on NEW FP, Dr. Nagamatsu reported higher efficacy for large HCC, other treatment until now. We performed implantation of transcatheter arterial port and treatment modified NEW FP (bolus injection 50 mg cisplatin and 250 mg 5-fluorouracil (5FU) day 1 and 8, 4 h continuous arterial injection 1250 mg 5FU day 1–5 and 8–12.). There was no side effect passed first course treatment. After second course modified NEW FP, the size of large HCC was reduced 50 % over. We confirmed modified NEW FP was effective for this case. We experienced the case of marked effect of modified NEW FP for the large HCC. A possibility that NEW FP of treatment will be one of choices was suggested as a way of treatment of a progress large HCC.

P-0703

Clinical features and antibiotic sensitivity to causative microorganisms of pyogenic liver abscess

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Aim/background: *E. Coli* is well known to be a most common causative microorganism of pyogenic liver abscess, but recent rise in *Klebsiella pneumoniae* and reports on multidrug-resistant microorganism raise the need for modification on selection of antibiotics.

Methods: The study included 92 patients diagnosed with pyogenic liver abscess from January 1, 2008 to December 31, 2014 in a single center. Patient's age, comorbidities, laboratory tests, location and size of abscess, causative microorganism confirmed from blood and abscess cultures, and antibiotic sensitivity were analyzed.

Result: The average age was 62.3 years, average duration of admission was 17.2 ± 9.5 days, and there were no differences in duration of treatment and antibiotic sensitivity by age, comorbidity and size of abscess. Abscess drainage resulted in 92.4 % (85/95) positive for culture, and *Klebsiella pneumoniae* was the most common microorganism (65.2 %) and *E. coli* was 15.2 %. Same microorganism cultured for both abscess and blood culture were 32.95 % (28/85) and among *Klebsiella pneumoniae*, two were positive for extended-spectrum beta-lactamase (ESBL) resistant, so switched antibiotic to imipenem. Abscess drainage and antibiotic treatment resulted in 96.7 % (89/95) cure rate and mortality rate was 3.3 % (3/92).

Conclusion: The most common microorganism of pyogenic liver abscess in this study was *Klebsiella pneumoniae* and some were multidrug-resistant ESBL positive *Klebsiella pneumoniae* consisting 2.1 % (2/95) of total cases. Therefore it is evident that very cautious approach is required when choosing antibiotics. It is very important when treating pyogenic liver abscess to drain abscess in early course of disease and culture to identify causative microorganism and obtain antibiotic sensitivity for evaluation of antibiotic treatment while administering broad-spectrum antibiotic.

P-0704

Two cases of spontaneous intramuscular hematoma survived in liver cirrhosis patients

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Spontaneous intramuscular hematoma with liver cirrhosis is a very rare but fatal complication, so accurate and timely diagnosis and appropriate treatment are necessary. We experienced two liver cirrhosis patients with a spontaneous intramuscular hematoma and report two cases of spontaneous intramuscular hematoma survived in liver cirrhosis patients. We reviewed 14 previously reported cases of spontaneous intramuscular hematoma in patients with liver cirrhosis and found out that alcoholic patients accounted for 92.9 % and the mortality rate was about 86 %, in spite of supportive care and aggressive treatments such as trans-arterial embolization and liver transplantation. Unlike other cases previously reported, all our patients survived. In our cases, patients were visit to the hospital within 6 h after symptoms occur, and then proper evaluations about symptom and accurate treatment like trans-arterial embolization, supportive care such as red blood cell and platelet and fresh frozen plasma transfusion were performed quickly, all patients were able to survive. The mortality rate of intramuscular hematoma in liver cirrhosis patients is very high. Also, no treatment method of intramuscular hematoma in cirrhosis has not been clearly established.

However, our cases suggested that an early visit to hospital allows early diagnosis and exact treatment like arterial embolization and final survival of patients. Thus, physicians should pay more attention in diagnosing and treating a spontaneous intramuscular hematoma in liver cirrhosis patients.

P-0705

A case of hepatic hemangioma with Kasabach-Merritt syndrome in an adult patient

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Hemangiomas are the most common benign tumors of the liver. They are generally asymptomatic, but giant hemangiomas can lead to abdominal discomfort, bleeding, or obstructive symptoms. Kasabach-Merritt syndrome is a rare but life-threatening complication of hemangioma, which is characterized by consumptive coagulopathy with large vascular tumors. More than 80 % of Kasabach-Merritt syndrome cases occur within the first year of life and are associated with either kaposiform hemangioendothelioma or tufted angioma. However, there are few reports of Kasabach-Merritt syndrome with giant hepatic hemangioma in adults and, as far as we know, no reports of Kasabach-Merritt syndrome with hepatic hemangioma treated with 1st line medical treatment only. The most important treatment for this syndrome is removal of the large vascular tumor by surgery or medical treatment; however, surgical treatment has limitations due to bleeding tendency or patient condition. We herein present a case of unresectable giant hepatic hemangioma with disseminated intravascular coagulopathy. The patient was a 60-year-old woman who complained of hematochezia, ecchymosis, and abdominal distension. She refused all surgical management and was therefore treated with systemic glucocorticoids and beta blockers. After 2 weeks of steroid therapy followed by a tapering dose, she responded partially to the treatment. Her laboratory findings and hematochezia improved. She was discharged on hospital day 33.

P-0706

Exophytic and pedunculated hemangioma of liver: a rare entity

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Entrance: Hemangioma is a common benign tumor, but its exophytic and peduncle form is a rare entity. Most hepatic hemangiomas remain asymptomatic and require no treatment. Herein we report a case of exophytic hemangioma of the liver.

Case E: 62 years old woman admitted for epigastric and upper right quadrant pain with chronic dyspeptic symptoms. Previously treated with PPI's for more than 6 months. Upper GI endoscopy showed normal findings. Patient detected with abdominal ultrasonography and computed tomography. Radiologic examinations revealed hepatic exophytic and pedunculated hemangioma in contiguity of gallbladder. Due risk of torsion and uncontrolled symptoms laparoscopic resection performed without complication. Post-op follow up at the 3th and 6th months patient was symptom free and need no medication.

P-0707

An unusual hepatic tumour in an patient with chronic hepatitis B

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Hepatocellular carcinoma (HCC) is the most common malignant hepatic lesion in patients with Hepatitis B-related cirrhosis. We report a case of an unusual hepatic tumour in a patient with typical imaging findings of HCC. A 53-year-old woman with HBeAg-negative chronic Hepatitis B was evaluated for incidentally-detected tiny echogenic hepatic nodules measuring 0.2–0.3 cm, and coarse hepatic echotexture suggesting early cirrhosis on surveillance abdominal ultrasonography. Prior ultrasonographs in the past 8 years had been normal. Repeat ultrasonography 3 months later revealed 3 new solid, hypoechoic nodules in both hepatic lobes, the largest measuring 2.2 cm in maximal diameter. Arterial enhancement and venous washout were demonstrated in all lesions on both contrast-enhanced computed tomography and magnetic resonance imaging, consistent radiologically with multifocal HCC. AFP was normal at 1.81 µg/L. Worryingly, the MRI liver also detected multilevel vertebral metastases. Tc-99 bone scan confirmed widespread metastases involving the ribs, thoracolumbar spine and pelvis. While chronic hepatitis B certainly confers risk for HCC development, we were concerned that such rapid tumour growth, widespread metastases early in the course of disease, and normal AFP level were atypical for HCC. Liver biopsy was therefore obtained, yielding a plasmablastic neoplasm consistent with ALK-positive diffuse large B-cell lymphoma. The patient was referred to a Haematology service and has since commenced chemotherapy, with mid-treatment imaging showing marked disease regression. This case highlights that in spite of characteristic HCC imaging findings and a typical risk profile, alternative diagnoses must be considered when clinical features are incongruent with the natural history of HCC.

P-0708

Extra-renal malignant rhabdoid tumour in an adult: a rare case of a liver tumor

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Malignant rhabdoid tumours (MRT) are rare, highly aggressive tumours usually found in kidneys of young children. Extra-renal MRT has been reported in other sites including the liver, pelvis, central nervous system, abdomen, heart and soft tissues. Although uncommon, most reported cases of extra-renal MRT of the liver in the literature are in paediatric patients. We report a case of a 51 year old male who presented with 2 months of cough, weight loss of 17 kg and dysphagia. He had Type 2 diabetes and hypertension and was a previous alcohol drinker. Clinical examination showed peripheral stigmata of chronic liver disease: spider naevi, hepatomegaly and ascites. Laboratory investigations showed normocytic, normochromic anaemia, cholestatic liver function test, hypoalbuminemia and raised CA19-9. Hepatitis screen, alpha-feto protein, carcinoembryonic antigen were normal. Gastroscopy showed an extrinsic mass in the stomach. Computed Tomography demonstrated hepatomegaly with multiple irregular hypoechoic/necrotic lesions in both lobes; the largest in segment 4 measuring 8.5 by 11.9 cm. A 3 × 3 cm left adrenal

mass, ascites, lymphadenopathy were seen at the paratracheal, subcarinal, coeliac, para-aortic and aorto-caval regions. Histology of the liver mass showed large round cells containing eosinophilic cytoplasm and hyperchromatic, pleomorphic nuclei with nucleoli. Intracytoplasmic hyaline inclusion was seen. Immunohistochemically, the cells were positive for vimentin, EMA and CD99 and negative for S-100 and desmin. Cytokeratin was, however, negative in this case. The patient was palliatively managed. Our case highlights this rare, aggressive disease in the liver and as to date this finding is the first described in a middle aged adult.

P-0709

Hepatobiliary cystadenoma which misdiagnosed as hydatid cyst: a case report

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Hepatobiliary cystadenomas are extremely rare hepatic lesions constituting less than 5 % of hepatic cystic neoplasia, usually originating from intrahepatic bile-ducts. Through the obstruction of bile ducts by the tumor itself or mucin secreted by the tumor the patient become symptomatic. A 61 year-old female patient admitted to the hospital with jaundice. She had surgery 2 times in the past because of hydatid cyst. Her AST:393, ALT:597, ALP:327, GGT:771, Total/direct bilirubin:4.1/3.3. At sonography, a 5 × 4 cm lobulated cystic lesion with echogenic septa was detected at the left lobe of the liver together with dilatation of the intrahepatic bile-ducts and reported as compatible with hydatid cyst. At MRI a multiloculated/multicystic lesion associated with biliary ducts and compressing portal vein was seen and found compatible with recurrent/residual hydatid disease. A 3 cm polypoid filling defect causing obstruction of the main biliary duct of the left lobe together with Komi Type-Ia pancreaticobiliary junction anomaly was detected at the ERCP. Patient operated with the diagnosis of cystic mass lesion. At surgery, a cystic mass lesion located at the left lobe and former operational area, obstructing and growing into the bile ducts was detected and left hepatectomy was performed. Pathological examination revealed hepatobiliary cystadenoma. Hepatobiliary cystadenomas are often seen in middle-aged women and have malignant transformation potential. These neoplasms require complete resection because of the risk of local recurrence. Cystadenomas/adenocarcinomas can be confused with common health problem hydatid cyst disease. Hepatobiliary cystadenomas should be kept in mind especially in patients with recurrence of hydatid cysts.

P-0710

Heterogeneity of histological features of benign hepatocellular nodular lesions

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Aim: Examining the heterogeneity of histological features of benign hepatocellular nodular lesions.

Materials: Ninety-one operated cases (142 nodules) of benign hepatocellular nodular lesions including focal nodular hyperplasia (FNH) and hepatocellular adenoma (HCA).

Methods: These lesions were immunohistochemically re-classified into FNH and HCA with four subtypes. Clinical backgrounds of these cases were also examined.

Results: Of these 142 nodules, four cases showed non-typical positive immunohistochemical staining patterns for glutamine synthetase (GS) and serum amyloid A (SAA), namely heterogeneity of immunohistochemical staining patterns. Case 1 had seven nodules, in SAA staining, two nodules were entirely negative, two nodules showed focally positive findings and three nodules showed entirely positive. Case 2 and Case 3 had a nodule that showed “nodule in nodule” structure. The inner nodule of Case 3 presented entirely positive GS pattern and nuclear accumulation of b-catenin. This part was diagnosed as b-catenin activated type HCA. Whereas the outside layer presented map-like pattern by GS staining. This part was diagnosed as FNH. Case 4 showed triple layered structure. The outer layer showed map-like pattern of GS. The middle layer showed diffusely positive GS pattern. The inner layer showed diffusely positive GS pattern with nuclear accumulation of beta-catenin. The nodule of Case 4 was morphologically diagnosed as HCC. The three layers were interpreted as FNH, HCA and HCC, respectively.

Conclusion: These cases may suggest a possibility of the multistep carcinogenesis of benign hepatocellular nodular lesions.

P-0711

The results of treatment of gastric cancer with liver metastasizes

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In 16 patients was performed palliative gastrectomy (GE) and in 32 distal subtotal resection (DSR). The metastasizes in both hepatic lobes are established in 60.4 % patients, in the dextral lobe in 25 % and in the left lobe in 20.8 % patients. Quantity of metastasizes from 4 up to 11 clusters, diameter from 0.6 to 4.5 cm. Depending on adopted tactics in treatment of liver metastasizes distributed on main (25 patients) and control (23 patients) groups. Distribution by the form and volume of the transaction in both groups were identical. In a main group 3–4 weeks after operation with the purpose of liquidation and depressing the growth of metastatic clusters was conducted long term endoarterial chemotherapy by installation the catheter into A. Hepatica Communis. Fluouracil 5 g and Doxorubicinum 60–80 mg injected, slowly with the special metering device during 120 h 2–3 times with an interval 1.5–2 months. In control group the same drugs in the same doses were entered system intravenously during 5 days. The treatment in 11 patients was repeated 2 and in 9 was 3 times.

Outcomes: The postoperative complications were advanced in 27 % patients, died 4. The full regressions of metastatic locuses, in both groups was not observed. The partial regression of metastasises in main group has compounded 68 %, the stabilization 32 %, development is not marked. In control group the partial effect is marked in

34.8 % patients, stabilization 39.1 %. The median lifetime of patients in main group has compounded 16.2 months and in control 11.4 months.

P-0712

A case of hematogenous metastatic small intestinal tumor of hepatocellular carcinoma

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The patient was an 86-years-old male, who was diagnosed hepatocellular cell carcinoma for the first time, 7 years ago. And he was treated with surgical and medical treatment. At this time, computed tomography revealed multiple liver metastasis recurrence and peritoneal dissemination, and we were introduced our hospital to resect a dissemination lesion. The operative findings showed two elastic soft dissemination lesions in mesentery, and these nodules performed exclusion of the mesenteric arteries and the veins. The dissemination nodules are exfoliated from a blood vessel and it was resected. Furthermore, a small intestine tumor was detected in 70 cm actinal side from terminal ileum. It is not exposed to a serosa, but it was present to make expulsion outside intestinal tract. And the tumor resembled dissemination nodules in a shape, therefore he was suspected with metastases to small intestine of HCC, and partial resection of small bowel was performed. Unidentified anemia was detected for a half year; we expected that the cause of anemia is a small intestine tumor. The pathological diagnosis was the peritoneal dissemination and metastases to small intestine of the hepatocellular carcinoma. The resected specimen of the small intestine revealed that a tumor showed a dark red, weak ulcer. Pathological examination revealed that clear nodules were formed from the mucosa outer layer to the sub serosa. That is reason, a small intestine tumor seemed to present with hematogenous metastasis than disseminated metastasis. The hematogenous metastasis of HCC is rare case, and we report it and review the literature.

P-0713

A case of small hepatocellular carcinoma with bile duct tumor thrombus

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A 74-year-old asymptomatic female was admitted to our hospital with liver enzyme elevation by biochemical examination of blood. Computed tomography revealed a nodular lesion in 22 mm size and peripheral biliary expansion in segment 8 of her liver. Endoscopic retrograde cholangiography showed a filling defect at the right hepatic duct of approximately 1.5 cm from right and left bifurcation. We considered a mass-forming cholangiocellular carcinoma and intra-ductal papillary neoplasm of the bile duct from these findings. She

underwent right lobectomy of liver after right branch of portal vein embolization. Histopathological examination showed a poorly differentiated hepatocellular carcinoma (HCC) of single nodular with extranodular growth type. The carcinoma has the growth in the bile duct. The most of bile duct tumor thrombus (BDTT) with HCC is found in advanced HCC and a small HCC with BDTT is rare.

P-0714

Antifibrotic activity of NK cells is impaired by TGF- β -dependent emperipolesis in liver cirrhosis

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Objectives: Natural killer (NK) cells have been implicated in inducing fibrosis remission in mice model; however, their anti-fibrotic immune characteristics and relevant regulatory mechanisms remain to be elucidated especially in liver cirrhosis (LC) patients with hepatitis B virus (HBV) infection.

Methods: Thirty-six CHB patients and 53 LC patients were recruited for this study. The frequency, phenotypes and functions of peripheral and intrahepatic NK cells were analyzed. In vitro co-culture was used to investigate the cell-in-cell structure (emperipolesis).

Results: NK cells from HBV-infected LC patients displayed a decreased frequency, activation status and anti-fibrotic activity as compared to CHB patients. In livers of LC patients in situ, NKp46+ NK cells were enriched in proximity to α -SMA+ area and were parallel increased with α -SMA+ cells within the periportal and lobular regions. These hepatic NKp46+ NK cells were either directly attached to or separated distributed with α -SMA+ hepatic stellate cells (HSCs). In vitro, NK cells displayed reduced cytolytic capacity against HSCs in LC patients compared to CHB patients. NK cells from HBV-infected patients have more potential to entered HSCs, form emperipolesis and display apoptotic status. Blockade of TGF- β pathway significantly enhanced NK cell-producing CD107a and IFN- γ production and partially prevented the emperipolesis of NK cells within HSCs. Finally, the anti-fibrotic activity of NK cells was correlated negatively with liver fibrosis scores in a longitudinal follow-up cohort of HBV-infected LC patients.

Conclusions: the impaired anti-fibrotic activity of NK cells was associated with liver fibrotic progression in chronic HBV infection through HSC-producing TGF- β and cellular emperipolesis.

P-0715

Deficiency of indoleamine 2,3-dioxygenase aggravates the development of CCl4-induced liver fibrosis

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Background: In the development of liver fibrosis, extracellular matrix is produced by activated hepatic stellate cells (HSCs) under the stimulation with pro-inflammatory cytokines, which are secreted by inflammatory cells. Recent studies indicated that indoleamine 2,3-

dioxygenase (IDO) suppresses inflammatory cells and induces immune tolerance. In the present study, we examined the role of IDO in the development of liver fibrosis.

Methods: Wilde type (WT) mice and IDO-KO mice were intraperitoneally administered with carbon tetrachloride (CCl₄) for the induction of liver fibrosis. Pathological grade of liver fibrosis was evaluated on Azan and Sirius red stained tissues and total collagen content was measured in the liver. The pro-inflammatory cytokine expression in the liver was examined using quantitative RT-PCR. The frequency and cell number of F4/80+ CD11b+ cells in the liver were analyzed by flow cytometer. Activated HSCs were observed by α -SMA staining. The fibrogenic makers in HSCs were evaluated using quantitative RT-PCR.

Results: CCl₄-induced hepatic fibrogenic areas were increased in IDO-KO mice. The total collagen was also increased in IDO-KO mice. The expression of TNF- α was up-regulated in IDO-KO mice compared with WT mice. The frequency and cell number of F4/80+CD11b+ cells were significantly increased in IDO-KO mice. The density of activated HSCs increased in the liver of IDO-KO mice compared to WT mice. The mRNA expression of ACTA2 and Col1a2 were up-regulated in IDO-KO mice. Conclusions: These results indicated that the deficiency of IDO aggravates the development of liver fibrosis. Thus, the enhancement of IDO expression in inflammation may suppress the liver fibrosis.

P-0716

Growth factor mobilized CD34+BMSCs rescue the loss of regenerative microenvironment in cirrhosis

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Background: Therapeutic effect of growth factor mobilized CD34+BMSCs has been shown in cirrhosis. However the underlying mechanisms is not clearly understood.

Aim: To study the cellular and molecular mechanisms of growth factor activities on liver regeneration in CLD patients.

Methodology: Patients with cirrhosis (N = 41) were administered growth factors week for 2 months and TJLB and hepatic vein samples were obtained before and after the therapy. Regenerative response was studied by immunohistochemistry (IHC) using CK19 and Ki67. Cellular microenvironment was analyzed by IHC by using cell type specific markers. Change in secretory microenvironment were analyzed by cytokine bead array in both responders and non-responders.

Results: Out of 41 cases 29 (70.73 %) showed cumulative response. Analysis showed increase in CD34+ (P = 0.001) cell recruitment, reduction in α -SMA (P = 0.036) and increase in Ki67 (P = 0.0001) as well as increase in CD163 (P = 0.06) M2-macrophage in responder group. Increased CD34+ cells was correlated with loss of α -SMA (p < 0.001, r = -0.640) and loss of α -SMA was correlated with increase in Ki67 (P = 0.011, r = -0.430) positivity. Analysis of cytokines and growth factors showed decreased in α -SMA were correlated with TGF β 1 (P = 0.002, r = 0.475), PDGF (p < 0.001, r = 0.520) and OSM (P = 0.004, r = -0.439), IL17A (p < 0.001, r = -0.544) and IL13 (p < 0.001, r = -0.745). Level of TGF β 1 (P = 0.029), and PDGF ab/bb (P = 0.01) were down regulated and TGFA (P = 0.01), OSM (P = 0.04) and IL13 (P = 0.033) were significantly up regulated in responders suggesting that decrease in myofibroblasts might lead to decrease in TGFB mediated inhibition of

hepatocyte replication while simultaneous increase in hepatotrophic growth factor TGFA and OSM might be responsible for increase in hepatocyte replication.

Conclusion: Growth factor mobilized CD34 + BMSCs restore the regenerative microenvironment in decompensated liver.

P-0717

Homocysteine deteriorates intrahepatic derangement and portal-systemic collaterals in cirrhotic rats

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In liver cirrhosis, the uneven performance of vasoactive substances, especially endothelin-1 (ET-1), nitric oxide (NO), and prostacyclin leads to increased intrahepatic resistance, angiogenesis, portal-systemic collaterals, and abnormal intra- and extra-hepatic vascular responsiveness. These derangements aggravate portal hypertension-related complications such as gastroesophageal variceal bleeding. The impaired collateral vasoresponsiveness to vasoconstrictors such as long-acting analogue of vasopressin (terlipressin) also adversely affects the treatment efficacy. Homocysteine, a substance implicated in cardiovascular diseases, has been found with influences on vasoresponsiveness and angiogenesis. However, their relevant effects in liver cirrhosis have not been surveyed. In this study, liver cirrhosis was induced by common bile duct ligation (BDL) in Sprague-Dawley rats. BDL rats received homocysteine or vehicle from the 15th to 28th day after BDL. On the 29th day, portal and systemic hemodynamics, mesenteric angiogenesis, portal-systemic shunting, and liver fibrosis were evaluated. The effects of homocysteine on intrahepatic and portal-systemic collateral vascular responsiveness were also evaluated by in situ perfusion models. The results showed that homocysteine enhanced hepatic vasoconstriction to ET-1 but decreased portal-systemic collateral vasocontractility to arginine vasopressin. Homocysteine downregulated hepatic phosphorylated endothelial NO synthase (p-eNOS) and p-Akt protein expressions and upregulated inducible NOS and cyclooxygenase-2 expressions in splenorenal shunt, the most prominent intra-abdominal collateral vessel. Homocysteine also increased portal-systemic shunting and hepatic collagen fiber deposition. Protein expressions of mesenteric angiogenic factors (PDGF, PDGFR β , p-eNOS) and hepatic fibrotic factors (collagen-I, α -SMA, TIMP-1) were upregulated by homocysteine. In conclusion, homocysteine is harmful to vascular derangements and liver fibrosis in cirrhosis.

P-0718

Human mesenchymal stem cell therapy is feasible for liver cirrhosis associated with HBV infection

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Background: The present study aimed to investigate the safety and clinical feasibility of human umbilical cord mesenchymal stem cells (UC-MSC) therapy for the decompensated liver cirrhosis patients associated with hepatitis B virus (HBV) infection.

Methods: 60 patients with HBV-related decompensated liver cirrhosis received conventional medical treatment combining with UC-MSC at a dose of $0.5\text{--}1.0 \times 10^6/\text{kg}$ body weight three times with 4-week interval. Other 120 patients with HBV-related decompensated liver cirrhosis received conventional medical treatment as controls. All participants were followed up for 96 weeks since transfusion of UC-MSC. The liver function and adverse events were evaluated during the follow-up period.

Results: No complications and side-effects were found in the UC-MSC treated patients during the 96-week follow-up period. The UC-MSC transfusions significantly increased cholinesterase, globulin and alkaline phosphatase, reduced the Child-Pugh scores during the follow-up period. However, there were no significant differences between two groups in alanine transaminase, total bilirubin, albumin, total cholesterol, and prothrombin activity.

Conclusions: UC-MSC transfusion is safe in the clinic and improves liver function and may serve as a novel therapeutic approach for patients with HBV-related decompensated liver cirrhosis.

P-0719

Identification of Axon guidance signaling pathway in hepatic stellate cells and liver fibrogenesis

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Background and aims: To identify the novel signaling pathway and key molecules that is involved in liver fibrogenesis by dynamic network analysis of liver transcriptomes.

Methods and results: Two experimental models of hepatic fibrosis were employed by intraperitoneally injection of diethylnitrosamine (DEN) or carbon tetrachloride (CCl₄) for 4 weeks. The liver samples were collected at 0, 6, 8 and 14 weeks post DEN treatment for RNA sequencing. The transcriptomic data associated different stages of liver fibrosis were applied to time-series analysis, followed by gene act network and pathway analyses. It was found that in the dramatically upregulated genes during liver fibrotic and cirrhotic stages, 48 were enriched in axon guidance signaling pathway ($P = 4.65\text{E}-07$, enrichment = 2.53), which includes genes that express the ligands (slit2, Sema4D), membrane receptors (Robo1 and 2, Eph and B, CXCR4, Plexin A4), downstream kinases (PAK1, 2,3 and 6, Gnai 1 and 2, Rock2, and Mapk3). By immunofluorescence staining in immortalized human and mouse hepatic stellate cell (HSC) lines LX-2 and JS1 in vitro, and in the CCl₄ induced fibrotic liver samples in vivo, Robo2, a key receptor mediating axon guidance signaling, was localized to the HSCs surface. The amount and localization of its protein was correlated well with fibrotic septa in fibrotic livers.

Conclusion: There are significant upregulation and activation of axon guidance signaling pathway in liver during fibrogenesis. This signaling may have important function toward HSC activation. The

study also revealed RoBo2 as a novel HSC surface marker and a potential therapeutic and diagnostic target.

P-0720

Inhibition of TGF-beta and TIMP1/2 by siRNA showed a different prevention of hepatic fibrosis

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Silencing the TGF-beta1, TIMP-1 and TIMP-2 with short interfering RNA to observe the expression of IFN-gamma, IL-4, IL-13 in hepatic fibrosis rats and to evaluate different roles of TGF-beta1, and downstream cytokines TIMP-1/2. The rats received carbon tetrachloride (CCl₄) by subcutaneous injections every three days for 8 consecutive weeks, and in the meantime they also obtained either siRNA (0.25 mg/kg) targeting TGF-beta1, TIMP-1 and TIMP-2, or saline, or a negative control siRNA plasmid by intraportal vein injection to the liver at the same pattern. The liver tissue from different groups were used to detect the expression of IFN-gamma, IL-4 and IL-13 by immunohistochemistry, western blot and real time PCR. The expression of Th1 cytokines IFN-gamma in the saline group (model group) and negative control siRNA plasmid group (control group) was significantly lower than the normal health group, and the expression of Th2 cytokines IL-4, IL-13 was significantly higher than the normal group. The expression of IFN-gamma in the TGF-beta1 siRNA group was significantly higher than the model group, while the expression of IL-4, IL-13 have no significant difference comparison to the model group and control group. The expression of IFN-gamma in the TIMP-1 siRNA group and the TIMP-2 siRNA group were significantly higher than the model group, and the expression of IL-4, IL-13 were significantly lower than model group. The difference of IL-4 and IL-13 expression between TGF-beta1 and TIMP-1/2 siRNA groups revealed the targets of TIMP-1/2 maybe a more suitable approach for liver fibrosis treatment.

P-0721

Enhancement of hepatocyte differentiation from human embryonic stem cells by Chinese medicine FZHY

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The Chinese medicine, Fuzhenghuayu (FZHY), appears to prevent fibrosis progression and improve liver function in humans. In this study, we employed FZHY to evaluate whether it enhances hepatocyte differentiation from human embryonic stem cells (hESC). After treatment with FZHY, albumin expression was consistently increased during differentiation and maturation process, indicating that FZHY might promote differentiation and maturation process. Expression of metabolizing enzymes and transporter were also increased in treated cells, indicating that the biotransformation function would likely be

increased. Importantly, we found that expression of mesenchymal cell markers and cholangiocyte marker were significantly reduced by treatment with FZHY. This suggests that one possible mechanism by which FZHY may promote hepatocyte differentiation and maturation is through inhibiting the formation of mesenchymal cells and cholangiocytes. Our hESC-derived hepatocytes (hEH) have shown proliferative capacity by co-expressing albumin and Ki67 even at a late stage of differentiation, and Edu-labelled flow cytometry results showed that the percentage of the Edu positive cells was increased in the treated cells. These results indicate that the enhanced proliferation involved hepatocytes rather than another cell type. Our investigations further demonstrated that the enhancement of hepatocyte differentiation and maturation is mediated through activation of Wnt and ERK signaling pathways and inhibition of the Notch pathway by FZHY. In conclusion, FZHY not only promoted hepatocyte differentiation and maturation, but also enhanced hEH proliferation. This demonstrates that FZHY treatment appears to enhance our efforts to generate mature hepatocytes with proliferative capacity for cell-based therapeutics and for pharmacological and toxicological studies.

P-0722

Collagen matrix modulates macrophage expression of MMPs in part by controlling cell shape

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Background and Objectives: Macrophages have a pivotal role in resolution of liver fibrosis, during which, macrophages undergo a switch in phenotype and up-regulate expression of Matrix metalloproteinase (MMP) 9, 12 and 13. However, the signals that regulate this phenotype switch remain to be fully determined. We hypothesized that alterations in cell shape may modulate the phenotype of macrophages in fibrotic liver. We therefore examined the effects of collagen on macrophage shape and MMPs expression in vitro, and investigated the underlying mechanisms with the aim of developing future anti-fibrotic therapies.

Method: Mouse bone marrow derived macrophages (BMDMs) were cultured on a monolayer of collagen I or plastic alone and the shape, size and MMPs expression determined. To clarify the relationship between effects on cell shape and MMPs expression, actin polymerization of BMDMs was assessed by phalloidin staining. BMDMs were then treated with Cytochalasin D and Rho family GTPase inhibitors to inhibit actin polymerization and determine the effect on MMPs expression.

Result: Culture on collagen I inhibited expression of MMP9, 12 and 13 by BMDM ($p < 0.05$) but stimulated actin polymerization ($p < 0.001$). Treatment with cytochalasin D resulted in the loss of actin polymerization ($p < 0.001$) and increased MMPs expression ($p < 0.001$). Similarly, the Rac1 inhibitor, NSC 23766, inhibited actin polymerization and increased MMP9 expression in BMDMs, while the Cdc42 inhibitor, ML141, increased MMP13 expression.

Conclusion: These results suggest that interaction with extracellular matrix, specifically collagen I, negatively controls macrophage expression of MMPs in part by activating actin polymerization. Hence, actin polymerization may be a target for anti-fibrotic therapy.

P-0723

In vivo expression of TIMP-1/2 siRNA promotes apoptosis of HSC and Smad protein expression in liver

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The regression of hepatic fibrosis (HF) is primarily due to the apoptosis of activated hepatic stellate cells (HSC). Mechanisms involved in the induction of HSC apoptosis are of interest to the development of therapeutic strategies to reverse fibrosis. This study aimed to investigate the effect of TIMP-1 and TIMP-2 siRNA on apoptosis and Smad protein expression in HSC in a rat model of hepatic fibrosis. After CCL4-induction of hepatic fibrosis, rats were treated with siRNA via tail vein injection. Eight weeks later, TUNEL- and alpha-SMA-stained liver tissue samples were analyzed using fluorescence microscopy. The number of double-positive stained cells was significantly higher in TIMP-1 and TIMP-2 siRNA-treated rats than in cells from HF rats and those treated with nonspecific siRNA as a negative control. Real-time PCR showed that mRNA expression of Smad2, Smad3, Smad4, and MMP-9 was markedly reduced in TIMP-1 and TIMP-2 siRNA-treated rats compared with HF rats and negative controls. Immunohistochemistry and western blot assay showed a marked increase in the expression of Smad2, Smad3, and Smad4 protein in the TIMP-1 and TIMP-2 siRNA-treated rats. TIMP-1 and TIMP-2 siRNA treatment increased the apoptosis of activated HSC, significantly inhibited the expression of Smad proteins, and increased MMP-9 mRNA expression in rats with CCL4-induced HF. These results suggest that siRNA technology might provide another approach to the development of anti-fibrotic therapies.

P-0724

The protective role of TGF- β induced upregulation of follistatin against mice hepatic fibrosis

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Background/aims: Follistatin (FST) is the antagonist of activin, a cytokine involved in the development of fibrosis. We provide the first evidence that FST is upregulated in TGF- β treated human hepatic stellate cell (HSC) and mice fibrosis.

Methods: LX-2 (human HSC line) cells were treated with recombinant human TGF- β 1 or medium vehicle. Microarray was performed to detect deregulated mRNA in TGF- β treated LX-2 cell. We performed qRT-PCR and Western Blot (WB) to validated candidate deregulated genes. Mice hepatic fibrosis were induced by Carbon tetrachloride (CCl₄), Thioacetamide (TAA) and bile duct ligation (BDL). siRNA targeting FST were delivered into mice using a hepatic specific liposome vehicle.

Results: We observed 220 up-regulated and 342 down-regulated genes in TGF- β treated cells in microarray analysis. FST was significantly upregulated in TGF- β treated cell in microarray analysis ($P < 0.05$). qRT-PCR and WB validated the upregulation of FST in TGF- β treated LX-2 cells ($P < 0.01$). FST upregulation was observed in the fibrotic livers of mice induced by CCl₄, TAA and BDL by qRT-PCR ($P < 0.01$ for mice in 3 models), WB and immunohistochemistry (IHC). Administration of siRNA targeting FST

significantly aggravated CCl₄ induced hepatic fibrosis, evidenced by increased upregulation of collagen 1 and SMA, exacerbated histological change and enhanced elevation of serum ALT level. FST was upregulated in hepatitis B and hepatitis C patients with different stages of fibrosis by IHC for FST.

Conclusions: FST is upregulated in TGF- β treated human hepatic stellate cell, mice fibrotic livers and patients with hepatic fibrosis and exerts protective role against hepatic fibrosis.

P-0725

CD147 in the immune response to liver injury in a novel conditional knockout mouse model

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Introduction: Chronic liver disease is characterised by inflammation that drives progressive fibrosis. The glycosylated transmembrane protein CD147 (a.k.a. basigin, EMMPRIN) expressed ubiquitously and is known to be a key mediator of inflammatory responses. We have previously demonstrated a pivotal role for CD147 in liver inflammation and fibrotic injury. CD147's exact role in the initiation, progression and resolution of liver inflammatory responses is unknown. We investigate the effect of selective CD147 knockout on immune cells and its effect in acute liver injury.

Materials and methods: Mice were generated by crossing CD147^{fl^{ox}} transgenic mice with Vav1-Cre transgenic mice. 6–8 week old male mice were given a 1.2 mL/kg carbon tetrachloride (CCl₄) for 72 h and 4 weeks. Tissue injury was assessed by LFT's, H&E and PSR staining.

Preliminary results: Mice with selective knockout of CD147 in immune cells show a significant decrease in both inflammatory infiltrate aggregates and hepatocyte necrosis in the liver in response to CCl₄, most pronounced around the portal vein. Ballooning and vacuolisation of hepatocytes were observed in the centrilobular areas of Vav1-Cre CD147^{fl^{ox}} mice in response to injury. No significant difference in collagen deposition was seen. A significant decrease in serum transaminases (ALT and AST) was observed between wild-type and knockout mice ($p < 0.03$).

Discussion/conclusion: Decreased immune infiltrate into the liver in Vav1-Cre CD147^{fl^{ox}} mice suggests that targeting CD147 may be a potential therapeutic in preventing intrahepatic inflammation. Together this suggests the CD147 expressed on infiltrating immune cells has a role in both the initiation and perpetuation of liver damage.

P-0726

Effect of decorin expressing adenovirus infected mesenchymal stem cells on cirrhosis rat model

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Background and aims: Decorin plays a protective role against fibrogenesis by modulating the degradation of the extracellular matrix (ECM), while bone marrow-derived mesenchymal stem cells (BM-MSCs) exhibit tropism for fibrotic areas and thus could be used as vehicles for targeted gene delivery. The aims of this study were to determine whether BM-MSCs infected with decorin-expressing adenovirus can ameliorate hepatic fibrosis progression and to investigate the molecular mechanisms of this process.

Methods: The effects of decorin-expressing adenovirus-infected BM-MSCs (DCN-MSCs) on hepatic fibrosis were examined in a rat model of thioacetamide (TAA)-induced cirrhosis. The effects of infection with decorin-expressing adenovirus and of incubation with the conditioned medium of DCN-MSCs on TGF- β signaling were analyzed in immortalized human hepatic stellate cells (HSCs).

Results: According to the Laennec fibrosis scoring system, cirrhotic livers from rats treated with DCN-MSCs exhibited histological improvement compared with cirrhotic livers from rats treated with control adenovirus-infected MSCs (CA-MSCs). DCN-MSCs treatment reduced hepatic collagen distribution, lowered the hydroxyproline content, and rescued liver function impairment in rats with TAA-induced cirrhosis. These protective effects were more potent with DCN-MSCs than with CA-MSCs. The upregulation of collagen-1, α -SMA, TGF- β 1, and Smad3 phosphorylation in cirrhotic livers was prevented by DCN-MSCs administration. Intriguingly, medium from cultured DCN-MSCs blocked both Smad3 phosphorylation and exogenous TGF- β 1 stimulated α -SMA synthesis in HSCs. **Conclusions:** DCN-MSCs exert strong protective effects against hepatic fibrosis by suppressing TGF- β /Smad signaling. Thus, treatment with DCN-MSCs is a potentially novel and efficient therapeutic approach for patients with intractable cirrhosis.

P-0727

Systemic administration of MSCs for hepatic ischemia reperfusion injury in a rat model

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Mesenchymal stem cells (MSCs) have been shown to reduce liver ischemia-reperfusion (I/R) injury and improve regeneration. The purpose of study was to investigate the difference in distribution of administrated MSCs in the liver, between ischemic injury area and non-ischemic area.

Material and methods: We used Fisher rats as donors of MSCs and recipients. Bone marrow derived MSCs were isolated from donor's femur. MSCs were labeled with fluorescent dye PKH26. Rats were divided into 4 groups: (1) I/R injury + MSCs transplantation, (2) MSCs transplantation only, (3) I/R injury + saline, (4) Sham group. I/R injury was performed by clamping vascular structures of the left

and middle lobes of the liver for 60 min. Right lobe was considered as a non-ischemic part respectively. Subsequently, 1.5×10^6 of MSCs or saline were administered via rat's tail vein. Thereafter, rats were sacrificed after day 1, 3 and 7 for analysis.

Results: Fluorescent microscopy assay showed the MSCs in both ischemic and non-ischemic parts of the recipient's liver. The number of cells was significantly higher in I/R injury + MSCs transplantation group compared to only MSCs transplantation group. Ki 67 staining showed that there was no significant difference in proliferation rate between I/R + MSCs and I/R + saline groups. Serum transaminase levels were also not different these groups.

Conclusion: After partial liver I/R injury, transplanted MSCs migrated to the both ischemic and non-ischemic parts of the recipient's liver equally. Considering the unique ability of the liver to regenerate, probably both parts of the liver received the signal for regeneration

P-0728

WT1 is the key regulatory of hepatic differentiation from bone marrow mesenchymal stem cells

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Background: BM-hMSCs have been known to differentiate into multi-lineage cell types and used for differentiated hepatocyte-like cells. The mesenchymal-epithelial transition (MET) plays as a key of cellular transdifferentiation programs, including wound healing and tissue regeneration. Wilms' tumor suppressor gene (WT1) controls transitions between the mesenchymal and epithelial state of cells. The purpose of this study is to clarify underlying differentiation mechanism and function of WT1 by screening the key factors in hepatic differentiation stem cells.

Methods: To detect the regulatory gene of BM-hMSC into functional hepatocytes, protein/DNA array was performed in BM-MSCs before and after differentiation. Hepatic differentiation of BM-hMSCs was evaluated using RT-PCR, western blotting, periodic acid-schiff staining, and a urea synthesis assay. To determine the effect WT1, after induction of hepatic differentiation from BM-hMSCs which were transfected with WT1 siRNA, identified the change of liver specific genes, transcription factors and MET markers.

Results: Here, we demonstrate that WT1 increases during hepatic differentiation of BM-hMSCs. Differentiated hepatocyte-like cells changed in morphology, function and hepatic gene expression. Also, the expressions of epithelial markers were increased, while the expressions of mesenchymal markers were decreased. In contrast, down regulation of WT1 reduced hepatic differentiation and increased the expression of mesenchymal markers but decreased the expression of epithelial markers.

Conclusions: In this study, we identified novel factors in the process of hepatic differentiation by MET. Our results demonstrate that BM-hMSCs may be a source of cells for liver regeneration and provide the mechanism of liver regeneration through MET process by the WT1.

P-0729

Sortilin deficiency affects fibrosis differently in hepatocyte vs. cholangiocyte models of injury

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Background/aims: The p75 neurotrophin receptor (p75NTR) is crucial for hepatic stellate cells (HSC) activation. This receptor can form heterodimers with sortilin, binding pro-nerve growth factor (proNGF) and inducing neuronal apoptosis. We investigated the role of neurotrophins and their receptors, in particular sortilin, in activation and apoptosis of HSC and in the development and regression of hepatic fibrosis.

Methods: Fibrosis was induced by three times weekly administration of thioacetamide (TAA) for 4, 6, 10 weeks (150 mg/Kg) and α -naphthylisothiocyanate (ANIT) (50 mg/Kg) twice weekly for 4 weeks to sortilin $^{-/-}$ mice and their wild type (WT) counterparts. Apoptosis was assessed using caspase 3 activity kit. Fibrosis was determined by Sirius red staining, hydroxyproline levels and qRT-PCR for fibrosis markers.

Results: HSC derived from sortilin $^{-/-}$ mice displayed increased activation and were less susceptible to NGF-induced apoptosis compared to HSC from wild type mice. Sortilin $^{-/-}$ showed increased hepatic fibrosis after 4 and 6 weeks of TAA administration, as demonstrated by increased Sirius Red staining, hydroxyproline levels and higher expression of collagen I and α -smooth muscle actin. Moreover, sortilin $^{-/-}$ mice had attenuated regression of fibrosis after TAA, due to reduced HSC apoptosis. In contrast, in a model of cholangiocyte injury induced by ANIT administration, sortilin-deficient mice exhibited dramatically attenuated fibrosis, and reduced early expression of pro-inflammatory cytokines TNF α and MCP-1 and attenuated ductular reaction.

Conclusions: Sortilin deficiency leads to increased liver fibrosis and attenuated regression in model of hepatocellular damage. However, in cholangiocyte injury, sortilin deficiency results in strongly attenuated fibrosis, due to reduced early inflammation.

P-0730

The activation of SH2 domain-containing phosphatase-1 by SC-43 ameliorates hepatic fibrosis

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Background: The signal transducer and activator of transcription 3 (STAT3) pathway is involved in liver fibrogenesis. We aimed to

investigate whether the activation of Src homology region 2 domain-containing phosphatase-1 (SHP-1) could downregulate phospho-STAT and further ameliorate hepatic fibrosis. Methods SC-43, a derivative of sorafenib and SHP-1 activator, was administered in hepatic stellate cells (HSCs) to investigate its antifibrotic activities, with special focus on the cellular apoptosis and SHP-1/STAT3 pathway. Furthermore, SC-43 was administered in two experimental hepatic fibrosis mouse models (CCl4 induction and bile duct ligation) to evaluate its antifibrotic activities *in vivo*. The SHP-1 expression was evaluated in chronic hepatitis B (CHB) patients with liver fibrosis for clinical implications. Results SC-43 promoted HSC apoptosis through STAT3 pathway inhibition. SHP-1 overexpression was associated with reduced cell viability of HSCs. The downregulation of SHP-1 by phosphatase inhibitors and siRNA rescued the SC-43-induced cell apoptosis. SC-43 increased SHP-1 activity by direct interaction with the N-SH2 domain of SHP-1, whereas the deletion of N-SH2 domain (dN1) and point mutation (D61A) of SHP-1 abolished the effect of SC-43 on SHP-1. Furthermore, SC-43 prevented liver fibrogenesis and ameliorated established fibrosis in both the CCl4 and bile duct ligation mouse models. In CHB patients with advanced fibrosis, SHP-1 was overexpressed in the fibrotic areas of liver, indicating SHP-1 might play a role in the process of fibrogenesis.

Conclusions: SC-43 ameliorates liver fibrosis through SHP-1 activation with subsequent STAT3 inhibition. SHP-1 phosphatase-directed STAT3 inhibition may represent a novel strategy for the discovery of antifibrotic drugs.

P-0731

Hepatotoxicity of industrial leachate; histopathology and heavy metal contents in liver

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Background and aims: Paper industry consumes a great amount of water as well as energy resulting in wastewater of dark brownish coloration with a strong foul smell. This leachate has some conventional pollutants viz, some toxic heavy metals, phenolics, and volatile organic compounds. The aim of present research was to assess the hepato-toxicity of leachate in Wistar rats.

Methods: 18 healthy male Wistar rats (240 ± 10 g) were randomly divided into three groups i.e. Control, Group 1 and Group 2. 4 ml/kg leachate was injected intraperitoneally to Group-1 while Group-2 received same quantity of 1:10 diluted leachate. Animals were euthanized after 24 h and liver tissues were excised for further studies. Data were analyzed using one way ANOVA.

Results: Liver metal contents carried out by flame atomic absorption spectrometer revealed a significant increase in level of cadmium and chromium in Group-1 compared to control. Histopathological analysis with H & E staining revealed leachate induced disruption in general micro-architecture of the hepatocytes, ruptured central vein, congestion of sinusoids, and disturbed morphology in Group-1, while vacuolization of cytoplasm, disrupted and congested hepatocytes, loss of polarity as well as necrosis in certain parts in Group-2 as compared to control.

Conclusion: Findings of the current research confirmed that leachate is a toxic industrial effluent which not only disturbs the liver metal contents it also disturbed the normal micro architecture of liver. Therefore, proper waste treatment of such water is required before its disposal.

Keywords: Heavy metals, Histopathology, Leachate, Toxicity, Waste water.

P-0732

Tacrolimus induced acute hepatotoxicity in wistar rats

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Background: Tacrolimus is widely used as a primary immunosuppressive agent after organ transplantation. It is eminent that the immunosuppressive effect of tacrolimus appears to depend on calcineurin inhibition. Owing to calcineurin inhibition, tacrolimus alters multiple biochemical processes and this may cause adverse side effects. The present study was aimed to investigate the histopathological alterations after tacrolimus induced acute inflammation.

Methods: Male Wistar rats (200 ± 25 g) were divided into five groups. Control group was provided with normal drinking water while tacrolimus was given to four experimental groups orally by preparing aqueous solution (3 mg/ml) and dissections were done after 6, 12, 24 and 48 h respectively. Livers were excised and histopathological alterations were assessed through haematoxylin and eosin staining and Masson's trichrome staining.

Results: Liver sections of control rats were devoid of any histological alterations having intact hexagonal structure with compact cells and pristine sinusoidal spaces. While after 6 h of dose ballooning degeneration of hepatocytes along with vacuole formation in zone 1 was observed. Sections after 12 h showed severe toxicity indicated by centrilobular necrosis, distortion of Sinusoidal spaces, congestion of hepatocytes and dilation of portal veins. 24 and 48 h time point exposed mild level of vascular damage, lobular architecture disruption, occurrence of plasma cells in sinusoidal spaces particularly nearby necrotic areas and inflammatory cells in the periportal areas. Masson's trichrome staining showed fibrosis limited to portal areas in 6 h treatment while after 12, 24 and 48 h time period fibrosis was also found to be bridging among the portal triads.

Conclusion: These findings indicate that tacrolimus may induce acute phase response resulting in hepatic damage.

P-0733

Therapeutic effects of ghrelin and leptin on experimental acute liver injury in mice

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Aim: Ghrelin and leptin are the hormones related to an appetite and nourishment state, and they are recently suggested to have immune-regulatory functions. In the current study, we tried to examine their therapeutic effects on liver injury and the mechanisms of immune-regulation using two murine models of acute liver injury, concanavalin A (ConA)- and carbon tetrachloride (CCl₄)-induced liver injury model.

Methods: C57BL/6 mice, 7- to 8-week-old females, were subjected to the experiments. The mice were injected intravenously with 15 mg/kg body weight of ConA diluted with PBS, or injected intraperitoneally

with 0.5 mL/kg body weight of CCl₄ diluted with olive oil. At 30 min after Con A or CCl₄ injection, 10 µg ghrelin or 40 µg leptin was intravenously administered into the mice.

Results: In the ConA model, the elevation of serum ALT levels and the histological damage in the livers at 8 h after ConA treatment were apparently ameliorated by the administration of ghrelin or leptin. In the CCl₄ model, the ALT elevation and the liver histology at 24 h after CCl₄ treatment also showed apparent amelioration by the administration of the individual hormones. In the analysis of cytokine/chemokine mRNA levels in the livers of the mice at 8 h after ConA injection, ghrelin significantly suppressed mRNA expression of TNF- α and IL-4, while leptin increased mRNA expression of IL-6 ($p < 0.05$).

Conclusions: Ghrelin and leptin exerted anti-inflammatory functions probably through the different mechanisms. The two hormones may become candidates of therapeutic reagents for acute liver failure.

P-0734

A dynamic relationship between cellular senescence and liver regeneration

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Aging is associated with an accumulation of senescent cells, a decline in the regenerative capacity and an increased risk of cancer development. To investigate the ability of senescent cells to influence tissue homeostasis we used a mouse model combining oncogene-induced senescence (OIS) and liver regeneration. Analyzing liver regeneration in the context of existing senescent cells, we detected a reduced proliferation rate, reduced mitotic index post PH and increased p21 positive hepatocytes. In wild type mice, senescent hepatocytes trigger senescence surveillance, leading to the elimination of these cells by the immune system and suppression of tumorigenesis. Even in immune-deficient mice spontaneous senescence escape is very inefficient. This indicates the importance of cellular senescence for cancer suppression. Surprisingly, already an acute liver damage led to efficient senescence escape and resulted in liver tumors. Transcriptome analysis reveals a set of genes which are significantly up- or down-regulated during senescence escape, implicating them to play a key role in this process and to be potential therapeutic targets. Immunohistochemistry also shows an expansion of NRas positive cells already at 30 days post PH. Taken together, our findings show on one hand the ability of the evolutionary conserved liver regeneration program to override the senescence program and on the other hand, the capability of senescent cells to attenuate the efficacy of the regeneration process. This is consistent with the decline in the regenerative capacity during aging and an increase in cancer incidence. It also supports the idea for an active role of senescent cells in both conditions.

P-0735

Protective effect of Ganshuang granules on liver cirrhosis by suppressing regulatory T cells

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Objective: To investigate the potential antifibrotic mechanisms of Chinese medicine Ganshuang Granules (GSG) and to provide clinical therapeutic evidence of its effects.

Methods: A cirrhotic mouse model was established by intraperitoneally injecting a mixture of CCl₄ (40 %) and oil (60 %) at 0.2 mL per 100 g of body weight twice a week for 12 weeks. After 12 weeks, GSG was fed to the mice for 2 weeks, and the mice were divided into low-, medium- and high-dose groups at doses of 1, 2 and 4 g/kg/day, respectively. Liver morphology changes were observed using Masson's trichrome staining and B-ultrasound. The regulatory T cell (Treg) frequency was determined through flow cytometry analysis. The expression of desmin, SMA, Collagen-I and IL-6 in liver were measured.

Results: Masson's staining and ultrasonography showed fewer fibrous connective tissues in the GSG-treatment group than in the spontaneous recovery group. The serum ALT, AST, HA levels were significantly ameliorated by GSG treatment (ALT: $F = 8.104$, $P = 0.000$; AST: $F = 7.078$, $P = 0.002$; and HA: $F = 7.621$, $P = 0.001$). The expression levels of collagen-I and SMA in the cirrhotic livers were also attenuated by GSG treatment (collagen-I: $F = 3.938$, $P = 0.011$; SMA: $F = 4.115$, $P = 0.009$). Tregs, which were elevated in the fibrotic livers, were suppressed by GSG treatment ($F = 8.268$, $P = 0.001$). The expression of IL-6 decreased in the cirrhotic livers after GSG treatment (IL-6: $F = 5.457$, $P = 0.004$). **Conclusions:** GSG promoted the resolution/regression of cirrhosis and restored liver functions in part by suppressing Treg cell differentiation, which may be mediated by hepatic stellate cells.

P-0736

Characterization of crosslinked porcine extracellular matrix as scaffold for hepatic bioengineering

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Xenogeneic extracellular matrix (ECM) holds great potential as bioactive support with regard to ex vivo organ regeneration. The use of crosslinking agents on ECM to stabilize the ultrastructure and enhance scaffold durability is constantly gaining interest. Although crosslinkage of xenograft matrix with glutaraldehyde was revealed to successfully reduce immunogenicity, optimizing the method of this strategy is needed before large-scale utilization on solid organ regeneration and clinic treatment. The aim of this study is to systematically compare the scaffold properties of porcine ECM crosslinked with different crosslinking agents (glutaraldehyde, genipin and quercetin). To this end, the mechanical properties, stability, abilities to induce immune cell invasion, neovascularization

characteristics of crosslinked ECM were evaluated. Both the ultimate tensile strength and max elastic modulus of decellularized porcine liver ECMs increased significantly after crosslinking. Enzymatic degradation assay also indicated that crosslinking after could protect liver matrices from enzymatic degradation. Glutaraldehyde and genipin were observed to remarkably reduce the migration and aggregation of human leukocyte subsets in a transwell migration study. And the histology staining of crosslinked ECM implants in a rat greater omentum implantation model showed relatively mild inflammatory reaction and intense signs of vimentin + fibroblast infiltration and CD31+ neovascularization in genipin crosslinking group. Implantation of crosslinked ECMs onto the chorioallantoic membrane of chicken eggs induced robust proliferation of endothelial cells and formation of blood vessel networks. In summary, our porcine liver ECM based on genipin crosslinking promises to be a preferable component of future implants, with improving pro-angiogenic properties and lower immunogenicity.

P-0737

TWEAK suppresses activated hepatic stellate cell senescence in vitro via regulating SIRT1 and p53

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Objective: To investigate the effects of tumor necrosis factor-like weak inducer of apoptosis (TWEAK) on the senescence of activated human hepatic stellate cells (HSCs) in vitro and to explore the potential mechanisms involved.

Methods: Human HSCs LX-2 senescence was evaluated by testing the activity of SA- β -Gal. The expression of Fn14 was examined with immunofluorescence and real-time PCR. The expression of SIRT1 and p53 was identified by real-time PCR and western blotting, and acetylated-p53 (ac-p53) was detected by western blotting. Primary rat HSCs were cultured to confirm the cell senescence and expression of TWEAK, SIRT1 and p53.

Results: LX-2 cells were maintained with 100 ng/ml of TWEAK for 24 h. The activity of SA- β -Gal was remarkably suppressed, indicating an inhibiting effect of TWEAK on HSCs senescence. The expression of Fn14 was significantly induced by TWEAK. The expression of SIRT1 was up regulated, detecting by real-time PCR and western blotting. The expression of p53 was not notably changed, whereas the ac-p53 was remarkably inhibited by TWEAK. Primary cultured HSCs were examined and showed that the activity of SA- β -Gal on day 11 was higher than that on day 7, and the mRNA of TWEAK, Fn14 and SIRT1 was all reduced on day 11 compared with that on day 7.

Conclusion: TWEAK inhibits activated HSCs senescence in vitro probably via up regulating SIRT1 expression and attenuating the acetylation of p53, which possibly reveals a novel potential mechanism of cell senescence negatively regulated by TWEAK.

P-0738

B cells may play a pathological role in hepatic inflammation in chronic liver diseases

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Background: B cells have been proven to promote liver inflammation and fibrosis in animal models. However, whether B cells may play a pathological role in chronic liver diseases (CLD) remains unclear.

Methods: A total of 93 patients with different types of CLD (including CHB, autoimmune hepatitis, alcoholic liver disease and primary biliary cirrhosis) were enrolled. Twenty-three normal liver tissues were included as controls. The degrees of hepatic inflammation and fibrosis of liver fibrosis in patients with CLD were graded using the modified histology activity index as described by Scheuer. B cells were determined by immunohistochemistry in the formalin-fixed, paraffin-embedded liver tissues of CLD patients.

Results: The density of CD20+ B cells was significantly increased in the livers of CLD patients compared to normal liver tissues ($P < 0.001$). In the CLD patients of different etiologies, the numbers of CD20+ B cells were all significantly increased ($P < 0.01$). CLD patients with higher inflammatory grades had significantly more CD20+ B cells infiltration in their livers compared to those with lower grades. However, intrahepatic CD20+ B cells were not positive association with liver fibrosis stages in these patients. Intrahepatic B cells were positively correlated with serum ALT ($r = 0.467$, $P < 0.001$), AST ($r = 0.310$, $P = 0.003$), GGT ($r = 0.247$, $P = 0.019$) and ALP ($r = 0.316$, $P = 0.002$) in CLD patients.

Conclusion: B cells may play a pathological role in hepatic inflammation in CLD patients caused by different etiologies. A clear understanding of the sources and functional roles of intrahepatic B cell infiltration in CLD should be further elucidated.

P-0739

Generation of transgene-free hepatocyte like cell with transposon mediated direct reprogramming

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Background: It has been indicated that somatic cells can be converted into hepatic lineage by overexpressing defined factors with direct reprogramming technique. Although there are various methods to deliver genes into cells for the purpose of overexpressing, genome-integrating viral vectors have a risk of mutagenesis whereas non-integrating viral vectors or episomal plasmids are transient and have low efficiency. Transposon is a unique DNA element which can be inserted into and removed from chromosomes in the presence of transposase, thereby gene integration during only the arbitrary period can be achieved. Here we demonstrate to generate transgene-free hepatocyte-like cells (TFiHeps) with non-viral transfection method using piggyback transposon vector for direct reprogramming.

Methods: To generate hepatocyte-like cells (iHeps), the vectors expressing Hnf4a and Foxa3 with RFP were transfected into mouse mesenchymal stem cells (mMSCs) along with transposase expressing

vector. Transgene-integrated MSCs with red fluorescence were cultured with differentiation medium. After generating iHeps, transposase expressing vectors alone were transfected, resulting in the achievement of TFiHeps lacking red fluorescence. Only TFiHeps were collected by fluorescence activated cell sorting and cultured as a form of spheroids. These spheroids were transplanted into damaged mice livers.

Results: Expression of albumin and mature hepatic functions were observed in the TFiHeps. The spheroidal TFiHeps retained hepatic functions as well as iHeps and repopulated in the mice liver.

Conclusion: We successfully generated TFiHeps. TFiHeps have a potential to be an alternative clinical choice for severe liver damage without the risk of mutagenesis.

P-0740

Impact of *Atp7b* deficiency on hepatic cholesterol metabolism and atherosclerosis in *Atp7b*^{-/-} mice

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Background: Wilson disease (WD) is caused by mutations in the copper transporting P₁-type ATPase *ATP7B* gene and results in copper accumulation and toxicity in liver and brain. In *Atp7b*^{-/-} mice copper accumulation in the liver leads to necro-inflammation followed by regeneration and neoplastic proliferation. Recently an unexpected link between hepatic copper overload and cholesterol metabolism was uncovered. Copper accumulation alters gene expression and cholesterol biosynthesis in hepatocytes resulting in reduced liver and serum cholesterol.

Methods: To further analyze these findings, *Ldlr/Atp7b*-DKO (double knockout) mice were generated and characterized. The *Ldlr*^{-/-} (low density lipoprotein receptor) mouse is a model for hypercholesterolemia and atherosclerotic plaque formation. After weaning at 4 weeks *Ldlr/Atp7b*-DKO and control mice were fed a cholesterol-enriched diet for 16 weeks. At 20 weeks mice were euthanized and serum lipids were quantified, histological analysis and microarray-based gene expression analysis were carried out in liver tissue. Atherosclerotic lesions were quantified in brachiocephalic artery and aortic root.

Results: In 20-week-old *Ldlr/Atp7b*-DKO mice expression of nine genes involved in cholesterol biosynthesis was reduced. *Ldlr/Atp7b*-DKO mice had significantly reduced serum cholesterol levels compared to *Ldlr*^{-/-}*Atp7b*^{+/+} control mice. *Ldlr/Atp7b*-DKO mice showed significant lower hepatic steatosis compared to controls. Atherosclerotic plaque formation was significantly reduced in female *Ldlr/Atp7b*-DKO mice (71400 ± 36500 μm²) compared to female controls (143300 ± 79000 μm²).

Conclusions: These findings in *Ldlr/Atp7b*-DKO mice further underline the relevance of the new link between copper and lipid metabolism and improve our understanding of the pathomechanisms of hepatic WD. This model may be important for the development of specific therapies to ameliorate WD progression.

P-0741

Involvement of endoplasmic reticulum stress in excess copper-induced hepatotoxicity

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Aims: Copper is an essential trace element, but excess copper is harmful to human health. Wilson disease is a genetic disorder characterized by excess copper deposition. It is known that the endoplasmic reticulum (ER) plays an important role in proper protein folding and accumulation of abnormal proteins disturbs ER homeostasis resulting in ER stress. However, copper-induced ER stress homeostasis disturbance has not been fully clarified. In this study, we analyzed the relationship between copper-induced ER stress and hepatotoxicity.

Methods: We used human hepatoma cell lines and an immortalized human hepatocyte cell line. The following materials were used: copper sulfate; acetyl-leucyl-leucyl-norleucinal and epoxomicin as proteasome inhibitors (PIs); bathocuproine disulfonate as a copper chelator; n-acetyl-l-cysteine as an anti-oxidant; 4-phenylbutyrate and ursodeoxycholic acid as chemical chaperones. We detected the reactive oxygen species using 2',7'-dichlorodihydrofluorescein. We analyzed copper-induced ER stress with phospho α -subunit of eukaryotic initiation factor 2 and X-box binding protein 1. Hepatocyte apoptosis was determined by detection of cleaved poly ADP-ribose polymerase and cleaved caspase 3. Furthermore, we examined cell proliferation with Ki67 and DNA double-strand breaks with γ H2AX.

Results: Excess copper induced not only oxidative stress but also ER stress. Co-treatment of copper and PI exacerbated copper-induced apoptosis. Furthermore, excess copper inhibited cell proliferation and induced pan-nuclear γ H2AX. Chemical chaperones ameliorated copper-induced hepatotoxicity in parallel with a decrease in ER stress.

Conclusions: Excess copper induced hepatotoxicity via ER stress. ER stress may play a pivotal role in Wilson disease, and chemical chaperones may have benefits in the treatment of Wilson disease.

P-0742

Make the best decision facing suspected progressive familial intrahepatic cholestasis in fetal cases

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Introduction: All varieties of progressive familial intrahepatic cholestasis (PFIC) as a hereditary autosomal recessive disease are caused by a defect in biliary epithelial transporters. Clinical manifestations include cachexia, growth deficiencies, cirrhosis and if not prevented leads to liver failure, which can only be treated by liver transplantation which in turn costs huge expenses and triggers considerable complications. This study probing on mutation of genes

helps to decide the birth/abortion of suspected babies.

Materials and methods: In this study in 2015, a 7 year-old boy with pathologically and clinically known PFIC, who had received liver transplantation, was examined by next generation sequencing (NGS) to find the mutations on effective trinary genes and by the Sanger method to discover mutations' exact location which were checked in his parents. In the 10th week of mother's 2nd pregnancy, the fetus genome was double-checked for Pre- Natal Diagnosis (PND) via Chorionic Villus Sampling (CVS) by molecular genetics specialists in the United Kingdom and Iran.

Results: Mother's molecular investigation demonstrated the heterozygote pathogenic mutation of c.134a>3 on the ATP8B1 gene. Molecular analysis of the fetus was not in consistence with his/her sibling's genetic mutation. Subsequently, the shared- decision was not to abort the baby.

Conclusion: Molecular scrutiny of trinary genes in suspected couples can be done by NGS, Sanger and CVS methods to prevent them from having children with PFIC or prevent the abortion of healthy babies.

Keywords: Progressive familial intrahepatic cholestasis; Molecular gene analysis; Next generation sequencing; Sanger; Pre-natal diagnosis; Chorionic villus sampling

P-0743

Mutation analysis of HFE gene in a small cohort of hereditary hemochromatosis cases from North China

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Hereditary hemochromatosis (HH) is the most common autosomal recessive disease in populations of northern European countries, and high frequency of C282Y mutation in Hemochromatosis (HFE) gene was demonstrated, with a low frequency of accompanied H63D mutation. Studies have also shown the HFE H63D homozygote mutation alone rarely cause the accumulation of iron. However, the mutation status of HFE gene in HH patients from China remains to be explored. We assessed HFE mutation status in a small cohort of HH patients from North China. Genomic DNA of 11 samples including seven sporadic HH cases and two HH cases from two families, were screened for mutation in all exons of HFE gene by Sanger sequencing. No C282Y or any other HFE mutation was observed in any of the investigated cases, except the H63D heterozygote mutation was identified in two HH cases: one sporadic HH case and one family HH case, indicating the possible causative effect of H63D mutation in the pathogenesis of HH. However, it is interesting that in the two samples from one family, heterozygote H63D mutation were identified in both the HH case and another family member without HH phenotype, suggesting the complexity of pathogenic role of HFE mutation, and that the main type of HH from North China may be non-HFE related. Our initial data imply that the mutation status of HFE gene is quite different among populations from different ancestry. Further study with more HH cases as well as biological function of the HFE mutation is urgently needed.

P-0744

Protective effect of clusterin on hepatic fibrosis

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Aims: Clusterin, an 80-kDa disulfide-linked heterodimeric protein and its expression is altered in various diseases including cholestatic liver diseases. Previous our study, clusterin is a protective effect on renal fibrosis. However, the role of clusterin on hepatic fibrosis is unknown. Here, we examined whether clusterin has the protective effect on hepatic fibrosis in vitro and in vivo.

Methods: In vitro study, we cultured human stellate cell (LX2) cells. Clusterin knockout (KO) and wild type mice were injected thioacetamide (TAA) for 8 weeks to induce hepatic fibrosis. We investigated the effect of clusterin on hepatic fibrosis by tail-vein injection using adenovirus-mediated over-expression of clusterin (Ad-Clu). Changes of fibrosis related gene with or without Ad-Clu in LX2 cells and animal study were determined by RT PCR and western blot analysis.

Results: Clusterin KO mice increased fibrosis and the expression of fibrosis related gene expression compared with the liver of control mice. TAA treatment accelerated hepatic fibrosis. However, Ad-clusterin by tail-vein injection attenuated TAA-induced hepatic fibrosis. In vitro study, Ad-Clu inhibits TGF & Beta stimulated collagen expression in LX2 cells. In addition, Ad-clusterin inhibited TGF & Beta -induced smad3 nuclear translocation.

Conclusion: This study shows that clusterin inhibits hepatic fibrosis in vitro and in vivo through downregulation of TGF & Beta-Smd3 signaling. The present study suggests that clusterin plays a critical role in the regulation of hepatic fibrosis.

P-0745

Salvia Miltiorrhiza enhances NK cell versus HSC effect via modulating PD-L1/PD-1 Axis

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Background: Natural killer (NK) cells killing activated hepatic stellate cells (HSCs) is confirmed both in patients and animal models. Nevertheless, anti-fibrogenic effects of NK cells are suppressed during advanced liver injury. High expression of programmed death 1 (PD-1) on NK cells and programmed death-ligand 1 (PD-L1) on HSCs contribute to liver fibrosis. Salvia miltiorrhiza (SM), a Chinese

herbal medicine, is effective in treating liver fibrosis, but the immunological mechanism remains unclear. Here, we tested the hypothesis that the anti-fibrotic effect of SM was associated with promoting activation of NK cells via modulating PD-1/PD-L1 axis.

Methods: Liver fibrosis was induced with carbon tetrachloride (CCl₄) and primary NK cells were isolated from C56BL/6 mice. NK cells were pre-incubated with SM and then co-cultured with HSCs. Effects of SM on NK cells and HSCs were investigated *in vivo* and *in vitro*.

Results: We found SM increased frequency of NK cells, enhanced activities of NKG2D and Nkp46 on NK cells and inhibited activation of HSCs *in vivo* and *in vitro*. Interestingly, SM boosted activities of NK cells including increasing expressions of NKG2D and interferon-gamma before or after co-cultured with HSCs *in vitro*. Besides, SM could partially antagonize ASGM-1-induced NK cell depletion and alleviate its cell functions. High expression of PD-1 on NK cells and PD-L1 on HSCs were also significantly decreased by SM administration *in vivo* and *in vitro*.

Conclusion: SM could enhance the NK cell versus HSC effect to reduce liver fibrosis via modulating PD-L1/PD-1 axis *in vivo* and *in vitro*.

P-0746

Antioxidative activity of Deferasirox (DFX) in iron-induced oxidative stress of hepatocytes

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Objectives: Iron is an essential nutrient. Iron is vital for almost all living organisms as it participates in a wide variety of metabolic processes, including oxygen transport, DNA synthesis, and electron transport. The overload of iron increase Fenton reaction and the formation of reactive oxygen species (ROS) such as superoxide anion (O₂⁻), hydrogen peroxide (H₂O₂) and the highly toxic hydroxyl radicals (HO⁻). These radicals are likely to cause lipid peroxidation and liver cell damage. Iron-induced oxidative stress has been considered to be an underlying mechanism of liver injury and development of hepatocellular carcinoma. Deferasirox (DFX) is a new oral chelator with high iron-binding potency and selectivity. DFX reduces serum ferritin and effectively reduces the concentration of serum alanine aminotransaminase (ALT). However, whether DFX reduces the concentration of free radicals to protect the liver cells remains unclear.

Materials and methods: The Hepa G2 cell line was used as the target cells. The Hepa G2 cells were treated with overload iron (100 μM ferric ammonium citrate; FAC) and different concentrations (50 μM, 100 μM, 200 μM) of deferasirox for 0, 18, and 24 h. The cell viability, cell apoptosis and cell radical concentration measure was measured using the WST-1 and flow cytometry.

Results: Overloaded FAC(100μM)reduced cell growth significantly. DFX(50 μM, 100 μM, 200 μM)could improve iron-reduced cell growth(CON 0.15 ± 0.04,FAC 0.01 ± 0.002, FAC+ DFX 50 μM 0.1 ± 0.03, FAC + DFX 100 μM 0.12 ± 0.04, FAC + DFX 200 μM 0.1 ± 0.04). DFX (50, 100, 200 μM)reduced iron-induced ROS formation from cells(CON 5.4 %, FAC13.6 %, FAC + DFX 50 μM 7.6 %, FAC + DFX 100 μM 6.9 %,FAC + DFX 200 μM 0.8 %)and cell apoptosis(CON 8.2 %,FAC14.0 %, FAC + DFX 50 μM 11.5 %, FAC + DFX 100 μM 6.5 %, FAC + DFX 200 μM 8.5 %).

Conclusion: These data suggest that overloaded FAC inhibited cell growth and lead to apoptosis through increased ROS formation. DFX may play a role in reducing damage of liver cells by reducing the level of ROS formation.

P-0747

Hepatic stem/progenitor Cells are Sox9 and AFP double positive in human cirrhosis liver

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Background: Chronic and long-term liver injury activates hepatic stem/progenitor cells residing in the canal of Hering to participate in liver compensation and restoration. The immune characteristics of hepatic stem/progenitor cells in cirrhotic human liver are still unknown.

Methods: Immunohistochemistry were used to analyze the expression of hepatic nuclear factor (HNF) 4&alpha, &alpha-fetoprotein (AFP), &beta-catenin, cytokeratin 19 (CK19), HNF1&beta, Sox9 and &alpha-smooth muscle actin (αSMA) in normal human liver and cirrhosis human liver.

Results: In normal liver, hepatocytes were positive for HNF4&alpha and the hepatocytes around central vein expressed &beta-catenin. Mature cholangiocytes were positive for CK19, &beta-catenin, and HNF1&beta in the normal liver, which indicates &beta-catenin is not a good marker to identify hepatic stem/progenitor cells. There are few cells stained positive for Sox9 (a transcription factor of hepatic stem/progenitor cells) in normal liver, while cirrhosis liver contained many Sox9 positive cells in the fibrous septa, i.e. hepatic stem/progenitor cells. Some of these cells formed small bile ductular, some were lined together, and the others were distributed as single cells. All these cells were surrounded by &alpha-SMA positive myofibroblasts. These Sox9 positive hepatic stem/progenitor cells were a heterogeneous population. Most of these cells stained positive for AFP, and the cells formed small bile ductular expressed CK19, &beta-catenin, HNF1&beta. All these Sox9 positive cells were negative for HNF4&alpha.

Conclusion: Sox9 and AFP double positive cells could be considered as hepatic stem/progenitor cells in cirrhosis liver.

P-0748

Retrospective analysis of an Ontario based diagnostic referral program using transient elastography

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Background and aims: In 2010, The Toronto liver Centre (TLC) created the first FibroScan[®] referral program in Ontario, and has since been serving the community. The aim of this analysis is to understand how Ontario physicians use fibroscan testing alongside the approved guidelines in the management and follow-up of chronic liver disease.

Methods: 5438 patients were referred to TLC from 2010 to 2014 for fibroscan testing. Referrals were assessed for: referring physician specialty & ethnicity, catchment area, patient demographics, disease etiology, fibrosis stage at referral and time between serial fibroscans.

Results: Physicians: 262 physicians referred patients for fibroscans; 63 % were General Practitioners, 29 % Gastroenterologist/Hepatologists, 3 % Infectious Disease, and 5 % others. Physicians were: 67 % Chinese, 13 % South Asian and 20 % Unknown.

Patient demographics: Fibroscan tests were reported for 5,437 patients, 58 % men vs. 42 % women. Median age was 54 years for women vs. 52 years for men. Patients were diagnosed with HBV (3399), HCV (866), NAFLD (836) and others (350), respectively. Catchment area extended to 25 cities within Ontario and 1 outside of Canada.

Fibrosis stage: Stages reported for patients were: 67.8 % F0 or F1, 6.4 % F3, & 10.3 % F4/cirrhosis.

Conclusions: Referrals from general practitioners comprised 63 % of our database; where 16.7 % of patients had liver fibrosis of F2 to F4, thereby qualifying those patients for treatment reimbursement. Furthermore, the large catchment area for this analysis despite the lack of financial coverage, signifies the broad acceptance of fibroscan testing amongst patients and physicians.

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Metavir Score	Total	Liver Condition					
		HBV	HCV	Fatty Liver	Alcohol	Autoimmune	Other Liver Condition
Sample Size	5437	3399	865	836	107	100	172
F0							
Count	1224	920	106	146	11	13	35
Column %	22.5%	27.3%	12.3%	17.5%	10.3%	13.0%	20.3%
F0-F1							
Count	1643	1222	166	174	20	28	42
Column %	30.2%	36.0%	19.2%	20.8%	18.7%	28.0%	24.4%
F1							
Count	821	505	128	153	10	14	17
Column %	15.1%	14.9%	14.8%	18.3%	9.3%	14.0%	9.9%
F1-F2							
Count	297	155	50	66	10	9	10
Column %	5.5%	4.6%	5.8%	7.9%	9.3%	9.0%	5.8%
F2							
Count	541	262	120	115	12	14	21
Column %	10.0%	7.7%	13.9%	13.8%	11.2%	14.0%	12.2%
F2-F3							
Count	171	72	41	39	4	6	10
Column %	3.1%	2.1%	4.7%	4.7%	3.7%	6.0%	5.8%
F3							
Count	128	76	52	40	3	5	5
Column %	3.3%	2.2%	6.0%	4.8%	2.6%	5.0%	2.9%
F3-F4							
Count	142	73	31	25	5	2	9
Column %	2.6%	2.1%	3.6%	3.0%	4.7%	2.0%	5.2%
F4							
Count	61	24	27	10	2	0	1
Column %	1.1%	0.7%	3.1%	1.2%	1.9%	0.0%	0.6%
Established cirrhosis							
Count	359	90	144	68	30	9	22

P-0749

Fibroscan: a novel modality; initial results from low cost tertiary care centres of Pakistan.

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Fibroscan is a novel non invasive test, which has proven to be effective in predicting fibrosis in liver disease (1). It is less expensive than liver biopsy, and it has not been associated with any side effects. (2). Objective: To assess the results of fibroscan in our population.

Study design: Cross sectional study in 337 patients attending Medilink clinics and Patel Hospital Karachi, Pakistan.

Results: In our study of 337 patients, there were 247 (73.3 %) males, age ranged from 13 –84 years with a Mean of 43 years. Main indications were chronic hepatitis 175 (52 %) patients and non-alcoholic fatty liver disease (NAFLD) 138 (40.9 %) patients. Rest 24 patients had various indications like alcohol abuse, Primary Sclerosing Cholangitis, and patients referred for assessment of liver fibrosis before chemotherapy. 142 (42.1 %) patients among this group had significant fibrosis (F3–F4). 124 (36.8 %) patients had (F1–F2) stage of fibrosis, while 71 (21.1 %) patients had F0 Stage. Among patients with chronic hepatitis 44.5 % had advanced fibrosis, while 38.4 % patients of NAFLD had advance stage of fibrosis. 41 % male while 45.5 % females had significant fibrosis on fibroscan.

Conclusion: Transient elastography is an established, non invasive modality for staging of liver fibrosis. In our study fibroscan showed high degree of fibrosis in 42 % of patients which is very alarming and highlight the need of early diagnosis and treatment in liver disease patients.

P-0750

Fibroscan—are 10 valid measurements mandatory?

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Background and aims: The manufacturer of Fibroscan set criteria for a reliable result of liver stiffness measurement (LSM): ≥ 10 valid measurements (VM) with ≥ 60 % success rate and IQR/median ratio ≤ 30 %. An experienced operator can usually tell after 5 VMs if the result will remain the same after 10. We aimed to establish when we can resume at less than 10 VM.

Material and methods: We analyzed the data of 2598 patients that had LSMs recorded in our database in an interval of 5 years, all respecting the above mentioned reliability criteria. We considered

fibrosis as significant if LSM >7.1 KPa and cirrhosis if LSM >14.5 KPa.

Results: For identifying significant fibrosis, if only one measurement would be done, the result would be discordant (as compared to 10 VM) in 244 cases (9.39 %). For 5 measurements the discordance occurs in 97 patients (3.73 %) and for 7 VMs in only 64 (2.46 %). For cirrhosis, for one measurement compared to 10 the results are discordant in 91 patients (3.5 %), for 5 VMs in 32 (1.23 %) and for 7 VMs in 15 patients (0.57 %). For 3, 5 or 7 VMs, the groups that had IQR/median ratio ≤ 30 % had a statistically significant better correlation with 10 VMs in the identification of significant fibrosis and cirrhosis. For cirrhosis, 5 measurements all the 32 discordant patients did not fulfill the IQR condition.

Conclusions: 5 VMs are enough to identify cirrhosis and have a good concordance with 10 VMs (96.2 %) in the identification of significant fibrosis if IQR/median ratio ≤ 30 %.

P-0751

ARFI elastography correlation with liver biopsy in the evaluation of staging of fibrosis

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Background and aims: Acoustic radiation force elastography (ARFI) is a novel technique for non invasive assessment of liver fibrosis. In comparison to transient elastography, ARFI can be done in obese patients and in multiple sites of liver. Aim of this study was to investigate the accuracy of ARFI imaging as a non invasive method for the assessment of liver fibrosis compared to histologic liver fibrosis score.

Materials and methods: A prospective comparison study of ARFI elastography was performed in a consecutive series of patients who underwent liver biopsy for the assessment of fibrosis. Mean ARFI velocities (m/s) were compared with Modified Ishak score (F0 to F4) for fibrosis in liver biopsy.

Results: One hundred and fourteen patients (Mean age 44 ± 13 , M/F = 78/36) with liver diseases (Cryptogenic = 39, Hepatitis B = 37, Non alcoholic fatty liver disease = 32, Autoimmune = 3, Hepatitis C = 1 and others = 2) underwent ARFI and liver biopsies at the same time period. The median ARFI (10 measurements per patients) velocities of our study populations range from 0.84 to 3.81 m/s. The spearman correlation coefficient between the median values of the ARFI and the histologic fibrosis stage of the Modified Ishak score was highly significant ($p < 0.001$) with $\rho = 0.7$. Area under the receiver operating characteristic curve for the accuracy of ARFI imaging was 0.81 for the diagnosis of advanced fibrosis (Fibrosis stage, $F \geq 3$).

Conclusion: ARFI imaging has strong correlation with the histologic fibrosis stage along with good accuracy for prediction of severe fibrosis.

P-0752

Noninvasive assessment of liver fibrosis using RTE and a novel serum marker: WFA⁺-M2BP

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Background: Wisteria floribunda agglutinin (WFA)-positive human Mac-2-binding protein (WFA⁺-M2BP) is a new marker of liver fibrosis. Our aim was to compare the reliability of serum WFA⁺-M2BP and real-time tissue elastography (RTE) in assessment of liver fibrosis in patients with chronic liver disease.

Methods: A total of 87 patients underwent contemporaneous liver biopsy, RTE, and measurement of serum WFA⁺-M2BP level. The diagnostic reliability of serum WFA⁺-M2BP and the liver fibrosis index (LFI) (calculated from RTE imaging features) was assessed in patients with advanced (F3–4) and minimal (F0–1) fibrosis, and receiver operating characteristic curves (ROCs) constructed.

Results: The area under the ROC curve (AUROC) for the LFI used to diagnose advanced fibrosis was greater than that of the WFA⁺-M2BP AUROC (0.873, 0.534), while the LFI AUROC for diagnosis of minimal fibrosis was lower than that of the WFA⁺-M2BP AUROC (0.620, 0.627). At a cut-off of 1.0, the WFA⁺-M2BP parameters were: sensitivity 28.6 %, specificity 93.5 %, positive predictive value 88.9 %, and negative predictive value 42 % for diagnosis of minimal fibrosis. In patients with stage F1 or lower, the LFI correlated with body mass index (F0: $r = 0.647$, $p < 0.01$; F1: $r = 0.748$, $p < 0.001$).

Conclusion: RTE is a reliable tool to detect advanced fibrosis, although it may overestimate advancement in obese patients. WFA⁺-M2BP measurement more accurately excludes advanced fibrosis.

P-0753

The correlation of RDW to liver and spleen stiffness in patients with chronic liver disease.

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Background and aims: The benefit of red cell distribution width (RDW) and liver stiffness measurements (LSM) by FibroScan[®]; have been validated the assessment of liver fibrosis in patients with chronic liver disease^{1–2}. This study explores the utilization of RDW in evaluating liver fibrosis in patients with disease or various etiologies. We also assessed whether RDW correlated to liver stiffness and spleen stiffness measurements (SSM) in patients with advanced fibrosis and portal hypertension.

Methods: 69 consecutive patients underwent liver (LSM) and spleen stiffness (SSM) measurements using transient elastography. A complete blood panel including: ALT, AST, GGT, ALP, BILI, HgB, Hct,

RDW, MCV, and Platelet count was completed at the time of fibroscan testing.

Results: 100 % success rate was achieved with the following number of patients: HBV(23), HCV(20), NAFLD(15), AIH(11). All patients had a mean: LSM (26.70kPa), SSM (34.26kPa), AST(47.85IU/mL), ALT(48.51 IU/mL) and Platelet (178 E9/L). RDW was predictive of both liver ($P = 0.005$) and spleen ($P = 0.001$) stiffness with overall mean of $P = 0.003$. In patients with LSM <12 kPa, mean RDW = 14.18 %, while those with LSM >12 kPa had mean RDW = 17.22 %. In comparison to those with LSM < 20 kPa^{3–4} where mean RDW = 20.46 %, and LSM >20 kPa with mean RDW = 14.27 %.

Conclusion: RDW positively correlates to liver and spleen stiffness in patients with chronic liver disease. Our data suggests that this correlation is valid up to a certain cut off value of LSM beyond which point RDW plateaus or even decreases. Further studies are needed to evaluate the benefit of serial measurements of RDW in patients with chronic liver disease over time.



DZ	RDW Mean (%)	Plat. Mean(145-400 E9/L)	LSM Mean (kPa)	SSM Mean(kPa)
AIH	15.29	228.18	22.79	45.24
HBV	13.68	230.47	25.40	29.14
HCV	14.83	243.39	30.44	37.08
NAFLD	14.33	246.5	22.99	31.9

P-0754

Diagnosis model of HBV related compensated cirrhosis by ultrasonography and transient elastography

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Background: Diagnosis criteria of HBV-related compensated cirrhosis still remains controversial. The aim of the study was to establish a simple and non-invasive diagnostic model for compensated cirrhosis on the basis of liver ultrasonography and transient elastography.

Methods: CHB patients with liver biopsy were enrolled in the study and decompensated cirrhosis was excluded. Compensated cirrhosis was defined as F4. According to previous studies, the markers including Platelet, Albumin, Cholinesterase, imaging of liver ultrasonography and Fibroscan were often used to evaluate cirrhosis. Statistical analysis was performed using sensitivity, specificity, accuracy, multivariate Logistic regression and ROC curve to compare the diagnostic performance of different models among combination of these markers.

Results: Totally 549 patients (86 %, F2–3 and 14 %, F4) were included. The following 5 variables were combined to 30 models (Accuracy: 0.25–0.80, AUROC: 0.50-0.74): platelet count <100,000/μL; albumin level <3.5 g/dL; Cholinesterase down; FS >12.4 Kpa; and surface nodularity. Multivariate Logistic regression showed that these variables were independently associated with compensated cirrhosis ($P < 0.05$). Especially ultrasonography combined with Fibroscan and platelet, and ultrasonography combined with Fibroscan and albumin have better diagnostic performance than other 28 models (Accuracy: 0.85, 0.86 respectively; AUROC: 0.76, 0.79 respectively). Youden index of the ultrasonography combined with Fibroscan and platelet models was relatively higher than others (YI = 0.40).

Conclusion: Our study showed that a simple and non-invasive model based of ultrasonography and Fibroscan has important value for early correct diagnosis of compensated cirrhosis. Especially after their combination with platelet or albumin, the diagnostic performance was highly improved.

P-0755

Further exploration of the effect of body positioning on spleen stiffness in cirrhotic patients

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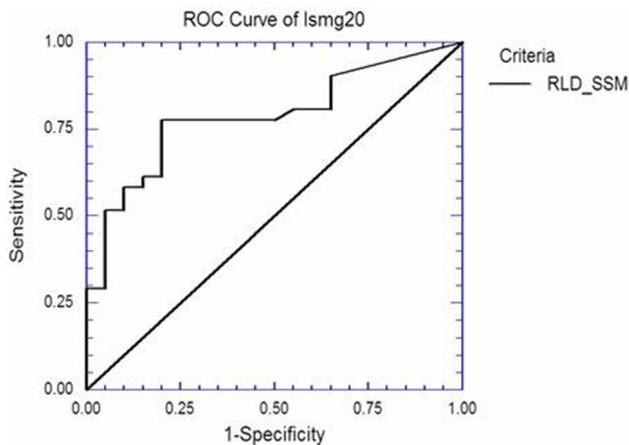
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Aims: We analyzed the effect of body position on patients with varying degrees of cirrhosis using transient elastography both in supine (S), and right lateral decubitus (RLD) positions. We further explored the effect of liver disease on spleen stiffness measurement (SSM) in cirrhotic patients.

Methods: 132 consecutive Liver stiffness (LSM) and SSM fibroscan tests were conducted on cirrhotic patients (Mean age 60.9/ BMI 30.7) in S and RLD positions. Patients were divided into 2 main groups; those in whom LSM <20.00 kPa(Group I), or >20.00 kPa(Group II) respectively (1–2). Group II was further divided based on disease etiology: Chronic HBV (B), Chronic HCV (C), NAFLD (F), and Autoimmune hepatitis (A).

Results: 100 % success rate was achieved with equal number of tests in S & RLD positions. *S-position:* Groups I & II had a mean SSM of 35.60 kPa & 35.70 kPa, respectively; $Pr = 0.988$, showing no statistical significance. *RLD-position:* Groups I and II had a mean SSM of 14.80 and 34.90 kPa, respectively; $Pr = 0.0003$; showing a strong statistical significance. *Group I:* SSM measured (kPa) in S and RLD-positions were as follows respectively: B(36.31,19.19), C(14.13,15.28), F(43.74,11.08) and A(54.56,12.82). *Group II:* SSM measured (kPa) in S and RLD-positions were as follows respectively: B(34.40,32.21), C (26.79,35.54), F(31.91,35.00) and A(55.73,36.93). Using T-test analysis for LSM of 20.00 kPa, the best cut-off value for RLD-SSM is equal to 20.90 kPa, yielding a SN = 0.77, and SP = 0.80.

Conclusions: Our findings suggest that SSM depends on the degree of cirrhosis and liver disease etiology. RLD position is superior in predicting increased LSM and portal hypertension in cirrhotic patients.



P-0756

Body mass index in Hepatitis B and C related liver disease; First Fibroscan based study from Pakistan.

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Hepatitis B and C are foremost cause of liver disease in Pakistan. Obesity is an additional factor leading to early development of Cirrhosis. Fibroscan is a new technique, allows non-invasive measurement of hepatic fibrosis. Prevalence of Hepatitis B and C in our population has inclined me to collect this data and is the first study of its type from Pakistan.

Objective: The purpose of this study was to evaluate fibroscan results of Chronic hepatitis B and C patients and compare results of high body mass index (BMI) >28 versus BMIs <28.

Study design: Cross-sectional study in 175 patients with chronic hepatitis B and C

Results: In our study there were 175 patients; male were 121 (69.1 %), age range 13–84 years, mean age 43.91 years. Mean BMI 25.41 kg/m², 127 patients had BMI <28 while 48 patients had BMI >28. 77 (44 %) patients had significant (F3–F4) fibrosis, while 89 (50.9 %) patients had (F1–F2) fibrosis and only 9 (5.1 %) had no fibrosis F0. Out of 127 patients having BMI <28, 36.2 % (46) patients had significant F3–F4 fibrosis, while 48 patients having BMI >28, 64.5 % (31) had significant (F3–F4) fibrosis.

Conclusion: In our study 44 % patients had F3–F4 fibrosis. 64.5 % patients having BMI >28; were found to have significant fibrosis as compared to only 36.2 % patients having BMI <28; (p-value of 0.0010). We conclude that patients with chronic hepatitis should have early assessment of liver fibrosis using Fibroscan in particular patients with high BMI, as this group carries higher risk of fibrosis progression.

P-0757

Transient elastography is not useful in the diagnosis of schistosomal hepatic peri-portal fibrosis

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Background/aim: Transient Elastography (TE) is a widely-used noninvasive measure liver stiffness. This study aimed to evaluate the diagnostic accuracy of TE in the diagnosis of schistosomal hepatic peri-portal fibrosis (SH-PPF).

Patients/methods: A total of 30 patients (mean \pm SD age 42.1 \pm 8.8 years) with pure schistosomiasis were included. Abdominal ultrasound (US) and upper gastrointestinal endoscopy were performed to all patients to assess for signs of portal hypertension and the presence a of varices as sequels of SH-PPF. TE (FibroScan) was done to determine liver stiffness. A cut-off value of >10.1 Kpa indicates advanced fibrosis (F3–F4).

Results: Splenomegaly was detected in 27(90 %) cases and was moderate to marked (>15 cm) in 19(63.3 %). Esophageal and gastric varices were found in 25 (83.3 %) and 4 (13.3 %) cases respectively. TE was successful in all patients, and the mean \pm SD liver stiffness was 9.4 \pm 5.5 Kpa (Range: 3.5–30 Kpa). F0–F2 was detected in 22(73.3 %) cases and F3–4 was detected in the remaining 8 (26.7 %) and EV and splenomegaly were detected in 18/25(81.8 %) and 19/27 (86.4 %) of cases with non-advanced fibrosis, and in 7(87.5 %) and 8(100 %) of cases with advanced fibrosis respectively (p = 0.71 & p = 0.27 respectively).

Conclusion: To our knowledge, this study shows for the first time that TE is not useful in diagnosis of SH-PPF and EV in patients with pure schistosomiasis. Whether this is applicable to other cases of pre-hepatic portal hypertension, such as portal vein thrombosis, congenital hepatic fibrosis, needs further investigation.

P-0758

Optimal liver stiffness values for diagnosis of liver fibrosis and cirrhosis

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Background: Liver stiffness measurement (LSM) is important for non-invasive assessment of fibrosis and cirrhosis in chronic liver disease (CLD). However, clear recommendations for the optimal cut-off LSM for clinical use remain lacking. We aim to validate the optimal cut-off LSM for significant fibrosis and cirrhosis in Singaporeans with CLD, and for specific etiologies of CLD. Methods: Prospective data from a multi-center study of CLD patients who underwent paired liver biopsy and LSM was analyzed using AUROC to determine the accuracy and optimal cut-off LSM to predict significant fibrosis and cirrhosis. A high quality cohort was selected by exclusion of invalid LSMs (success rate <60 %, IQR/M >0.3), BMI >30 kg/m², ALT >5 \times ULN and biopsy length <15 mm.

Results: Of 481 subjects recruited from two tertiary centers in Singapore, 322 subjects fulfilled the pre-defined quality criteria. Mean age was 49.4 \pm 12.3 years with 55.6 % males. CLD etiology was chronic hepatitis B(CHB) in 49 %, non-alcoholic steatohepatitis(NASH) in 16 % and chronic hepatitis C(CHC) in 12 %. AUROC of LSM was 0.775(95 % CI: 0.724–0.826) for significant fibrosis and 0.810(95 % CI: 0.738–0.882) for cirrhosis. In the overall cohort, optimal cut-off LSM was 9 kPa for significant fibrosis and 13 kPa for cirrhosis. The optimal cut-off LSM for individual CLD etiologies was 9 kPa for significant fibrosis and 12 kPa for cirrhosis in CHB and CHC and 11 kPa for significant fibrosis and 15 kPa for cirrhosis in NASH.

Conclusions: This study defines the optimal cut-off LSM levels for chronic viral hepatitis and NASH that are validated in a local cohort. Cut-off LSM levels are higher in NASH than CHB and CHC.

P-0759

FibroTouch is good for evaluation of liver fibrosis in Chinese patients with chronic viral hepatitis

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Aim: To explore the clinical application and related factors of FibroTouch in the diagnosis of liver fibrosis in patients with chronic viral hepatitis through comparison of FibroTouch, APRI and FIB-4, and to identify whether FibroTouch is more accurate and safe in diagnosis of liver fibrosis.

Methods: A total of 146 patients with chronic hepatitis B or C were performed liver biopsy and underwent LSM using FibroTouch. Serum ALT, AST and TBIL were tested by enzymic method with automatic biochemistry analyzer. Blood platelet counts were detected by automatic blood cell analyzer. APRI and FIB-4 were calculated. The diagnostic values of FibroTouch, APRI and FIB-4 for liver fibrosis degree were calculated and compared by AUROCs. The related factors of LSM were analyzed by Spearman analysis.

Results: There was significant correlation between LSM and histological fibrosis ($r = 0.76$, $P = 0.000$). The AUROC of LSM for $S \geq 2$, $S \geq 3$ and $S = 4$ was 0.87, 0.93 and 0.98, respectively, which was significantly higher than APRI (0.66, 0.68 and 0.73) and FIB-4 (0.73, 0.75 and 0.78). On Spearman analysis, LSM was positively correlated with age, ALT, AST, TBIL ($\geq 2 \times$ ULN) and the grade of liver inflammation ($r = 0.22, 0.52, 0.49, 0.54$ and 0.62 , respectively) but negatively with PLT ($r = -0.40$), (all $p < .$)

Conclusion: FibroTouch is a convenient and reliable approach for diagnosis of liver fibrosis in patients with chronic viral hepatitis. It is superior to APRI and FIB-4 for noninvasive diagnosis of hepatic fibrosis, and age, high level of ALT, AST and TBIL ($\geq 2 \times$ ULN) were independent predictors of LSM inaccuracy.

P-0760

Predictive value of MELD, APRI, and FIB-4 for mortality and development of HCC in cirrhotic patients

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Background and aim: Liver cirrhosis increases the risk of mortality and development of hepatocellular carcinoma (HCC); therefore, determining the predictors associated with mortality and HCC development are of vital importance. Several indices have been widely used to predict mortality and/or HCC development in patients with liver related diseases. The purpose of this study was to investigate the predictive value of proposed indices on mortality and HCC development in cirrhotic patients.

Methods: The medical records of 404 liver cirrhosis patients admitted to Konkuk university hospital from 2005 to 2009 were retrospectively reviewed. Excluding 60 patients who could not be followed, the data of 344 patients were analyzed.

Results: During the follow-up, 55 patients developed HCC and 125 died. New HCC patients had significantly lower CP score, MELD, and MELDNa levels than non-HCC patients. While no significance was found in APRI and FIB-4, non-HCC patients had significantly shorter follow-up duration than HCC patients. Those who died had significantly higher CP score, MELD, MELDNa, APRI and FIB-4 than living patients. Among all patients, those with MELD levels greater than the 14.50 cut point had protective effects against new HCC (OR = 0.385, 95 % CI = 0.165–0.899), and FIB-4 greater than the 4.50 cut point had significant risk (OR = 3.329, 95 % CI = 1.427–7.771) associated with new HCC. Furthermore, FIB-4 greater than the 9.50 cut point was significantly associated with mortality (OR = 2.738, 95 % CI = 1.168–6.419). **Conclusions:** FIB-4 was predictive of both new HCC and mortality. Thus, FIB-4 may have more predictive value for death and HCC development in cirrhosis patients.

P-0761

Acoustic radiation force impulse elastography (ARFI) in the diagnosis of hepatic fibrosis.

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ARFI is a new way of assessing liver fibrosis. In a prospective study we evaluated utility of ARFI in 171 consecutive patients undergoing liver biopsy and assessed misclassification rate of fibrosis in patients with elevated serum bilirubin or SGPT, ascites and patients with BMI more than 25 kg/m². **Material & method:** 171 consecutive patients (mean age 46.04 ± 13.34 years, males 60 %) undergoing liver biopsy were evaluated for ARFI by Acuson S2000, Siemens ultrasonography machine. Indications for liver biopsy were autoimmune liver disease (38), alcoholic liver disease (13), non-alcoholic fatty liver disease (24), cryptogenic liver disease (10), non-cirrhotic portal hypertension (29), viral hepatitis (17) and miscellaneous (40). Liver biopsy was graded by single pathologist blind folded by Metavir classification. ARFI value was correlated with fibrosis stage on liver biopsy. ARFI and fibrosis were compared with respect to serum bilirubin, AST, BMI, ascites, steatosis and esophageal varices.

Results: Mean ARFI score with interquartile range were 1.70 ± 0.46 (1.33–1.99) in stage 0, 1.92 ± 0.56 (1.45–2.16) in stage 1–2 and 2.49 ± 0.60 (2.07–2.98) in stage 3–4 fibrosis. Mean ARFI values were significantly higher in stage 3 and 4 fibrosis as compared to fibrosis stage 0 and 1 and 2 ($p < 0.001$). ARFI values were misclassified in patients with bilirubin more than 10 mg/dl, ALT more than 300 IU/L and ascites.

Conclusion: ARFI was reliable predictor of advanced fibrosis but could not differentiate stage 0 from early fibrosis. ARFI values were not reliable in bilirubin more than 10 mg/dl, ALT more than 300 IU/L and ascites.

P-0762

Noninvasive assessment of liver fibrosis using shear wave elastography in patients with CLD

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Background: The aim of the present study using shear wave elastography (SWE) was to evaluate the correlation between the liver stiffness measurement (LSM) and the liver fibrosis stage and to evaluate the accuracy of the LSM in comparison to the measurement of serum fibrosis markers (APRI, FIB-4 and platelet) in the prediction of significant and advanced fibrosis.

Methods: We consecutively analyzed 100 patients with chronic liver disease (CLD), 70 with hepatitis C virus and 30 with nonalcoholic fatty liver disease.

Results: The LSMs for each stage of fibrosis were as follows: 1.43 ± 0.21 m/s in F0 ($n = 30$), 1.54 ± 0.25 m/s in F1 ($n = 36$), 1.66 ± 0.32 m/s in F2 ($n = 8$), 2.03 ± 0.23 m/s in F3 ($n = 14$), and 2.23 ± 0.21 m/s in F4 ($n = 10$). A steady stepwise increase in the elasticity was correlated with the staging of liver fibrosis ($p < 0.0001$). The diagnostic accuracy of the LSM in the prediction of stages F >2 , F >3 and F4 was 0.901 (95 % CI: 0.843–0.961, $p < 0.0001$), 0.956 (95 % CI: 0.928–0.984, $p < 0.0001$) and 0.952 (95 % CI: 0.926–0.977, $p < 0.0001$) respectively. The cut-off values of the LSM in stages F >3 and F4 were 1.81 m/s with 96.2 % sensitivity and 85.1 % specificity and 2.01 m/s with 98.7 % sensitivity and 89.8 % specificity, respectively.

Conclusions: SWE was significantly correlated with the severity of liver fibrosis and was useful for predicting significant and advanced fibrosis. The level of accuracy was comparable to that achieved with the measurement of APRI, FIB-4 and platelet in CLD.

P-0763

The agreement of transient elastography (TE) and acoustic radiation force impulse imaging (ARFI)

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Background and aims: In the last 12 years liver elastography has been intensively studied and became a customary method for the evaluation of the liver fibrosis. In this study we aimed to assess the concordance between ARFI and TE in the evaluation of the liver fibrosis.

Patients and methods: 137 patients with chronic viral hepatitis were enrolled in the study. ARFI and Fibroscan were performed the same day and blood samples for ALT, AST, GGT, Albumin, platelet count and alpha-fetoprotein were collected. For TE we used the cut-offs: 5.5, 7.1, 9.5 and 14.5 KPa for F1, F2, F3 and F4 respectively. For ARFI we used 1.14, 1.34, 1.61 and 2 m/s for the same stages.

Results: 57 patients had hepatitis B and 80 hepatitis C. 82 (59.8 %) of the patients were females. ARFI and TE showed a good correlation, with $r = 0.579$ and $p < 0.0001$ when values were compared and $r = 0.706$, $p < 0.0001$ when corresponding stages were compared. A value of over 1.35m/s has 82.1 % sensitivity and 82.8 % specificity in the identification of significant fibrosis ($F \geq 2$ Metavir as identified by TE) (AUROC = 0.816, CI 95 % = 0.700–0.932). ARFI over 1.98 m/s has 82.4 % sensitivity and 82.4 % specificity (AUROC = 0.888, CI 95 % = 0.810–0.965) for cirrhosis (TE staged). ARFI showed no concordance with Fibroscan for the identification of F0 and F1 (AUROC 0.172 and 0.353).

Conclusions: ARFI and TE show a good agreement in the identification of significant fibrosis and cirrhosis, but the two methods are not concordant for F0 and F1.

P-0764

Value of VTTQ measurements for the risk assessment of HCC development in viral hepatitis patients

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Purpose: To evaluate the value of measuring shear wave velocity evoked by acoustic radiation force impulse (VTTQ) for the risk assessment of hepatocellular carcinoma (HCC) development in patients with viral hepatitis B (HBV) or C (HCV).

Methods: VTTQ was measured three times in each of the four liver segments in 45 HBV and 55 HCV patients, including 11 and 22 HCC cases, respectively. The results were statistically evaluated.

Results: The median VTTQ was 2.67 and 1.23 m/s in patients with and without HCC, respectively, with the difference being statistically significant ($p < 0.001$). The area under the receiver-operating characteristic curve for VTTQ in the differentiation of HCC cases from non-HCC cases was 0.900 (95 % confidence interval [CI], 0.96 to 1.00). Multivariate analysis showed that VTTQ (odds ratio [OR], 24.7; 95 % CI, 6.15 to 99.0; $p < 0.001$) was an independent explanatory variable for HCC presence.

Conclusions: These results suggest that VTTQ measurements implemented with acoustic radiation force impulse are useful to evaluate the risk of HCC development in a viral hepatitis cohort.

P-0765

The association between NAFLD fibrosis score, FIB4 index and serum vitamin D

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Background: This study aimed to evaluate the association of serum vitamin D value and NAFLD fibrosis score FIB4 index.

Material and methods: From August 2014 to August 2015, a community-based cohort study was performed in the north-eastern region of Taiwan. The inclusion criteria of NAFLD group were non-alcohol drinking, non-viral hepatitis subjects with elevated ALT (>36 U/L), r-GT (>26 U/L) and presence of fatty liver on abdominal ultrasonography. Subjects with normal abdominal ultrasonography, ALT, rGT and non-alcoholic, non-viral hepatitis were included in the control group. This study applied the fatty liver index (FLI) for steatosis evaluation and NAFLD fibrosis score, FIB4 index for fibrosis evaluation.

Results: Finally, 292 subjects were defined as NAFLD group and 252 subjects were control group. The mean age was 56.3 years. Subjects in NAFLD group had higher percent of IR and MetS than subjects in

the control group (IR 39.7 % vs. 7.5 %; MetS 41.8 % vs. 16.7 %, $p < 0.001$). FLI analysis revealed 61.6 % (180/292) subjects with index >60 (steatosis). NAFLD fibrosis score analysis revealed 80.5 % (235/292) NAFLD subjects with score <-1.455 (low degree fibrosis, F0–2). Vitamin D value was positively correlated with the NAFLD fibrosis score (linear regression analysis, unadjusted co-efficiency $B = 2.36$, 95 % CI = 1.57–3.16, $p < 0.001$) and FIB4 index ($B = 5.97$, 95 % CI = 4.25–7.69, $p < 0.001$).

Conclusion: NAFLD subjects have a higher percent of MetS and IR than normal control subjects. Most NAFLD subjects have liver steatosis but no advanced fibrosis status. Serum vitamin D value is positively correlated with NAFLD fibrosis score and FIB4 index.

P-0766

Using non-invasive APRI, FIB4-score and liver stiffness to identify cirrhosis in the community

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Aims: Using non-invasive aspartate aminotransferase/platelet ratio index (APRI), fibrosis-4 score (FIB-4) and liver stiffness measurement (LSM) to identify cirrhotic elders with chronic virus hepatitis in the community.

Methods: In Yujing, a mountain township in southern Taiwan, those residents who were screened as positive hepatitis B surface antigen and/or anti-hepatitis C virus before were invited to this study. All participants underwent blood tests, ultrasound and Fibroscan[®] examinations. Liver cirrhosis was identified based on the ultrasound.

Results: Among 481 responders, 281 (58.4 %) were chronic hepatitis B (CHB) patients, 188 (39.1 %) were chronic hepatitis C (CHC) patients and 12 (2.5 %) were dual hepatitis patients, had a mean age of 61.6 ± 12.2 years. Comparing serologic AST, ALT, APRI, FIB4, and LSM, CHC patients had significant higher level than CHB patients (47.4 IU/L, 46.3 IU/L, 0.9, 3.4 and 8.4 kPa in CHC patients and 36.3 IU/L, 36.9 IU/L, 0.5, 1.9 and 6.3 kPa in CHB patients, respectively). Nineteen cirrhosis patients in CHB and 23 cirrhosis in CHC were confirmed by the ultrasound. To predict cirrhosis, the areas under receiver operating characteristic curves (AUROC) for FIB4 was 0.881 (95 % confidence interval [CI], 0.831–0.931; $p < 0.001$), which was compatible to that of LSM value (0.863; 95 % CI, 0.799–0.927; $p < 0.001$), and APRI (0.836; 95 % CI, 0.769–0.902; $P < 0.001$). The best cut-off points related to cirrhosis were 2.8 for FIB4, 8.8 kPa for LSM and 0.6 for APRI, respectively.

Conclusions: In elder patients with chronic viral hepatitis in the community, using non-invasive serological FIB4, APRI or liver stiffness measurement could well predict ultrasound-identified cirrhosis.

P-0767

Significant relationship between liver stiffness and waveform patterns in the hepatic vein

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Aim: The aim was to examine the relationship between liver elasticity and waveform patterns in the hepatic vein (HV) in chronic liver diseases.

Methods: This prospective study consisted of 103 subjects (male 53, female 50, age 60.1 ± 11.3 years), 44 chronic hepatitis and 59 cirrhosis. The HV waveform findings on Doppler ultrasound, which were classified based on quantitative measures, were evaluated with respect to the clinical findings including liver stiffness measurement (LSM) value by FibroScanTM (Echosens, Paris, France) and portal pressure.

Results: There were three waveform patterns, monophasic in 14 (13.6 %, 5 chronic hepatitis, 9 cirrhosis), biphasic in 58 (56.3 %, 15 chronic hepatitis, 43 cirrhosis) and triphasic in 31 (30.1 %, 24 chronic hepatitis, 7 cirrhosis). The LSM value was significantly lower in the triphasic pattern group (10.9 ± 8.0 kPa) than in the monophasic pattern and the biphasic pattern groups (32.8 ± 22.6 kPa, $P < 0.001$ and 25.5 ± 18.0 kPa, $P < 0.001$, respectively), showing no significant relationship with portal pressure. The diagnostic ability for cirrhosis presented by the highest area under the receiver operating characteristics curve was 0.922 (81.4 % sensitivity and 90.9 % specificity; best cut-off value, 16.9 kPa) by LSM, and 1.000 (100 % sensitivity and 100 % specificity; best cut-off value, 19.4 kPa) by LSM combined with the monophasic pattern. **Conclusions:** This study demonstrated close linkage between liver stiffness and HV waveform findings. It may provide a better understanding and stimulate wider application of Doppler measurement in the HV in chronic liver disease.

P-0768

Change of liver cirrhotic patients during 15 years: Retrospective multi-center study in Korea

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Background/aims: Introducing of vaccine and antiviral agents for HBV, cause of liver cirrhosis seems to be changed as time goes on. We aimed to evaluate the changes of character of liver cirrhosis in Daegu-Gyeongbuk province in Korea during 15 years.

Methods: We reviewed retrospectively medical records of 15,539 patients of 5 university hospitals in Daegu-Gyeongbuk province from 2000 to 2014. The data were collected from KCD-6 codes related with hepatic fibrosis and cirrhosis, evaluated as each groups with 5-year interval.

Results: 1. Among 15,539 liver cirrhotic patients, men were 74.6 %. Mean age were 56.33 ± 14.28 for men, 60.66 ± 15.39 for women ($p = 0.015$). The numbers of patients were 5429, 5168, 4942 at 2000–2004, 2005–2009, 2010–2014. 2. The numbers of patients were 4113, 3824, 3660 for men, 1316, 1344, 1282 for women. Mean ages

were 56.74 ± 15.67 , 56.29 ± 13.11 , 55.90 ± 13.79 for men, 60.808 ± 15.92 , 61.48 ± 13.84 , 59.62 ± 16.34 for women in each time periods. 3. HBV was significantly decreased to 50.9 %, 44.2 %, 37.1 % ($p = 0.000$). HCV has no significant changes to 5.2 %, 6.6 %, 6.7 % ($p = 0.875$). Alcohol and NASH were increased to 31.5 %, 34.4 %, 36.9 % ($p = 0.005$), 2.5 %, 4.0 %, 5.6 % ($p = 0.000$) 4. Common causes of admission were variceal bleeding, ascites, hepatic encephalopathy. Variceal bleeding was significantly decreased to 25.7 %, 24.2 %, 22.2 % ($p = 0.000$), Ascites has no significant change to 21.4 %, 17.8 %, 21.1 % ($p = 0.072$), hepatic encephalopathy was decreased to 8.0 %, 7.2 %, 7.0 % ($p = 0.01$) 5. According to endoscopic findings, esophageal varix was decreased to 46.0 %, 38.0 %, 29.7 % ($p = 0.01$). Gastric varix was also decreased to 20.2 %, 17.5 %, 14.1 % ($p = 0.008$) 6. Among those who have been diagnosed with hepatocellular carcinoma during this period, HBV was decreased to 61.4 %, 58.0 %, 48.4 % ($p = 0.000$), HCV has no significant changes to 7.4 %, 7.3 %, 8.6 % ($p = 0.072$), NASH was increased to 1.4 %, 4.0 %, 6.8 % ($p = 0.000$) as cause of HCC. **Conclusion(s):** The etiologic burden of liver cirrhosis during 15 years in Daegu-Gyeongbuk province in Korea is changing. HBV has been decreased, alcohol and NASH are emerging as more important cause of liver cirrhosis. So we have to focus on patients' care and education for abstinence from alcohol intake. Similarly, we need more attention to diagnose and screen for NASH.

P-0769

Early diagnosis and biological markers in advanced liver damage of different etiologies

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Background: Chronic liver disease is a global health burden. Furthermore, the prevalence of liver disease may be underestimated due to the lack of early diagnostic strategies.

Aim: To assess the stage of liver damage (LD), main etiologies and biological markers (BMs) associated with advanced LD in West Mexico.

Study design: In a cross-sectional/analytical study, 427 subjects with risk factors for LD (Group I) and 130 clinically-diagnosed cirrhotic patients (Group II) were enrolled. Liver fibrosis was staged as F0–F4 by transitional elastography (TE) and BMs were assessed.

Results: Overall ($n = 571$), HCV infection ($n = 211/37$ %), alcohol ($n = 188/33$ %) and NASH ($n = 148/26$ %) were the main etiologies. In Group I, TE staging was F0 ($n = 115/27$ %), F1 ($n = 121/28$ %), F2 ($n = 73/17$ %), F3 ($n = 33/8$ %) and asymptomatic cirrhosis (F4) ($n = 85/20$ %). By LD etiology, F1–F3 was more prevalent in HCV ($n = 114$) than in NASH ($n = 55$) and alcoholic ($n = 47$) patients ($p < 0.05$). In contrast, in 215 cirrhotic patients ($n = 85$, Group I plus $n = 130$, Group II), alcohol abuse and HCV were mainly prevalent. Five BMs were associated with advanced LD (F3–F4): BM1 (OR = 3.599, 95 % CI 1.79–7.25), BM2 (OR = 3.309, 95 % CI 1.61–6.78), BM3 (OR = 3.929, 95 % CI 1.41–10.91), BM4 (OR = 3.887, 95 % CI 1.61–9.36) and BM5 (OR = 3.065, 95 % CI 1.50–6.26) ($p < 0.01$). AUCs were 0.69 (95 % CI 0.61–0.77, $p < 0.01$), sensitivity (66 %) and specificity (69 %). PPV (72 %), NPV (66 %), accuracy (0.43) and probability (98 %).

Conclusions Early diagnosis of fibrosis with TE was feasible among patients with risk factors for LD: 27 % were without fibrosis, 53 %

had F1–F3 and 20 % had asymptomatic cirrhosis (F4). Fibrosis staging prevalence differed by etiology. BMs could detect advanced LD regardless of disease etiology.

P-0770

Natural course and etiology of end-stage liver disease in Turkey

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Background and aim: The aims of this study were to define etiology and natural course of end-stage liver disease of 1654 cirrhotic patients.

Materials and methods: This was a multi-center retro/prospective study. A total of 1654 patients diagnosed with end-stage liver disease (ESLD), who were applied in the Liver Disease Outpatient Clinic were include. Cirrhosis was defined clinically and histologically when available. The median follow-up period was 39 months.

Results: Hepatitis B virus (HBV) was the most common cause of ESLD (38.2 %), followed by hepatitis C virus (HCV) (18.1 %), cryptogenic (15.7 %), fatty liver disease (12.8 %), autoimmune liver diseases (6.3 %), hepatitis delta virus (4.2 %) and others (%4.7). During admission, while 64.3 % of the patients had compensated disease, %35.7 were decompensated. 46 % were Child-Pugh class A, 46 % class B, 8 % class C. Median MELD score was 11.7. Ascites was the most common sign of the decompensation (61.4 %), followed by variceal hemorrhage (16.1 %) and hepatic encephalopathy (17.2 %). 5.3 % patients had diagnosed with hepatocellular carcinoma (HCC). During the follow-up period, 25.8 % of the compensated patients were progressed to decompensated stage, 62 patients developed de novo HCC. HCC occurred more frequently in patients with HBV-induced cirrhosis (76.8 %) and followed by HCV-induced cirrhosis (15.3 %). %2.9 of the patients underwent liver transplantation. The overall mortality was 9.0 %.

Conclusion: HBV infection keeps the leading cause of the ESLD and HCC in Turkish population. Hepatic decompensation and HCC may still develop, though at a lower rate in natural course of disease but the prevalence of HCC is decreasing currently.

P-0771

Possible role of hepatitis B virus in the etiology of cryptogenic cirrhosis**Beytullah Yildirim², Gulsen Isikli², Beytullah Yildirim¹, Ibrahim Goren¹, Talat Ayyildiz¹, Mustafa Kaymazli¹, Ahmet Bektas¹**¹Department of Gastroenterology, Ondokuz Mayıs University, School of Medicine, Samsun, Turkey; ²Department of Internal Medicine, Ondokuz Mayıs University, School of Medicine, Samsun, Turkey**Introduction and purpose:** Cryptogenic cirrhosis(CC) is a term used for patients whose etiology has not been determined although known causes of cirrhosis have been researched. The purpose of our study is to determine the frequency of HBV infection in patients with CC, to compare the obtained data with social studies conducted in our country and to assess the possible role of HBV in CC.**Material and method:** 170 patients who came to Ondokuz Mayıs University, Faculty of Medicine, Department of Gastroenterology between July 2005 and January 2015, who were 18 and older. They were included in this study after diagnosed CC. The patients' CC diagnoses were confirmed, their Hepatitis B tests were assessed.**Results:** Of the 170 patients diagnosed with CC, 91 were female and the mean age was 61 ± 14 years (range 18–89). While the HBV markers of 74 patients (43,5 %) were negative, 13 patients (7,7 %) were Anti-HBs (+) but were not checked for HbC total, 53 patients (31,2 %) were Anti-HBs (+) and Anti-HBc total (+), 16 patients (9,4 %) were Anti-HBs (+) and Anti-HBc total (–) and 14 patients(8,2 %) were Anti-HBs (–) and Anti-HBc total (+). The rates of previous HBV in patients with CC were isolated anti HbC total positive and Anti-HBs positive and it is obvious that it will be at least 40 % with the inclusion of patients whose Anti-HBc total were not checked.**Conclusion:** In our study, the rates of HBV history in patients with CC were high when compared with the data of the society. This situation supports the thought that HBV infection may have a role in the etiology of CC.

P-0772

Distinct circulatory miRNA profiles between cirrhotic patients infected with hepatitis B and C virus**Chau-Ting Yeh, Ya-Hui Huang, Kung-Hao Liang, Rong-Nan Chien, Tsung-Hui Hu, Chee-Jen Chang**

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Background: Hepatitis B virus (HBV) and hepatitis C virus (HCV) are two major etiologies of chronic hepatitis and liver cirrhosis. MicroRNAs are important gene regulators in chronic hepatitis. To date, the microRNA-mediated molecular pathogenesis of liver cirrhosis remains elusive.**Methods:** We recruited 330 cirrhotic patients infected with HBV (N = 220), HCV (N = 93), or both (N = 17). Plasma levels of 28 hepatocellular carcinoma-related, circulatory microRNAs were quantified. The receiver operating characteristic curves were used to evaluate the classification performance of microRNA in distinction between HBV and HCV related cirrhosis. The subjects were then randomly divided into training (N = 220) and validation (N = 110) cohorts. The generalized iterative modeling method was used to produce a classification score, calibrated by use of the Youden's index, for classifying patients with distinct etiologies.**Results:** Distinct circulatory microRNA profiles were found between HBV and HCV infected cirrhosis patients. Seven microRNAs were included to formulate a score to differentiate between patients with different etiologies in the training cohort, which included miR-21, miR-30c, let-7g, miR-15a, miR-122, miR-221 and miR-30b. The score achieved an area under the curve of 61.1 % and a significance level of 0.007 in the training cohort. The score distributions of the HBV and HCV groups remained significantly different in the validation cohort (Mann-Whitney P = 0.017). **Conclusions:** Distinct circulatory microRNA profiles were found between liver cirrhotic patients with HBV and HCV etiologies. This result indicated that different hepatitis viruses likely evoked distinct sets of microRNAs for gene regulation during the long-term courses from chronic hepatitis to liver cirrhosis.

P-0773

Baseline liver stiffness measurement can predict liver related events in chronic liver disease**Yu Jun Wong, Pik Eu, Jason Chang**

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Introduction: Liver stiffness measurement (LSM) reliably predicts liver fibrosis and cirrhosis in chronic liver disease (CLD). However, it is unknown whether baseline LSM can predict liver-related events (LRE) such as esophageal varices (EV), ascites, portal vein thrombosis (PVT) and hepatocellular carcinoma (HCC). We aim to evaluate the accuracy of baseline LSM for predicting LRE in CLD.**Methods:** Patients who had baseline LSM performed from 2005 to 2013 at our centre were prospectively monitored for development of LRE. Accuracy of baseline LSM for predicting individual LREs was assessed using AUROC. Cut-off LSMs to predict LRE were chosen based on maximal (>90 %) specificity.**Results:** 295 patients (median age 57.6 years, 68.5 % males) were recruited. Etiology of CLD was chronic hepatitis B in 70.5 %, 12.5 % chronic hepatitis C and 16.9 % non-alcoholic fatty liver disease. 50.5 % of the cohort had paired liver biopsy with baseline LSM (50.3 % METAVIR F0/F1, 37.6 % F2/F3, 12 % F4). LRE developed in 16(5.7 %) patients (8 EV, 5 ascites, 4 PVT, 8 HCC) over a median follow-up period of 5.4 (0.2–9.7) years. LSM was accurate for predicting ascites (AUROC 0.92, 95 % CI:0.85-0.99), PVT (AUROC 0.94, 95 % CI: 0.86–1.00) and HCC (AUROC 0.88, 95 % CI: 0.82–0.95) but not EV (AUROC 0.68, p = NS). Baseline LSM >17 kPa was >90 % specific for predicting eventual development of ascites, PVT and HCC with sensitivities of 60 %, 75 % and 38 %, respectively.**Conclusion:** Baseline LSM >17 kPa has high specificity for predicting development of ascites, PVT and HCC in patients with CLD. This may be helpful to risk-stratify CLD patients for closer monitoring for early detection of LREs.

P-0774

Reversion of HBV-related liver fibrosis/cirrhosis starts from septal area assessed by SHG images**Yameng Sun¹, Xiaoning Wu¹, Yuanyuan Kong¹, Qinmei Wu¹, Lin Wang¹, Bo Feng², Yimin Mao³, Jiyao Wang⁴, Lungen Lu⁵, Jilin Cheng⁶, Yongpeng Chen⁷, Jihong Sun⁸, Peng Hu⁸, Xue-en Liu⁹, Jialing Zhou¹, Dongyang Sun¹, Xiaojuan Ou¹, Jidong Jia¹, Hong You¹**

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Background and aims: Fibrosis and cirrhosis is known to be reversible after suppression of hepatitis B virus (HBV). However, the quantitation assessment and dynamic changes of collagen features during reversion of HBV-related fibrosis/cirrhosis still remain unknown.

Methods: Chronic hepatitis B patients were undergone liver biopsy before and after 78 weeks entecavir-based antiviral therapy. Liver biopsy samples were stained with hematoxylin-eosin/Masson and imaged by second harmonic generation/two photon excitation fluorescence (SHG/TPEF) microscopy which can quantify, locate and identify characteristics of portal, septal and fibrillar collagen.

Results: A total of 371 patients were prospectively enrolled in this multicenter study. After 78 weeks antiviral therapy, the fibrosis reversion (Metavir scoring system decrease ≥ 1 unit) rate was 67.4 %, 47.1 % and 40.0 % in F2, F3 and F4 patients respectively. Of the 30 paired biopsy samples which were imaged by SHG/TPEF microscopy, 14 (46.7 %) had fibrosis reversion. Among the 42 collagen features from portal, septal and fibrillar area, the changes of 6 collagen features (including string length, string width, number of strings, number of short strings, number of long strings and number of thin strings) correlated with fibrosis reversion ($P < 0.001$) and all were from septal area. Compared with baseline, these collagen features decreased 63.6, 65.2, 62.6, 60.2, 69.8 and 66.2 % in reversion patients after 78 weeks therapy respectively.

Conclusions: Reversion of HBV-related liver fibrosis appears to start in septal area, instead of portal and fibrillar.

P-0775

Dose serum urotensin-II Have role on pathogenesis at compensated and decompensated liver cirrhosis?

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Aim: Urotensin-II is a potent vasoconstrictor hormone. In this study aimed to investigate, urotensin-II possible role in the pathogenesis of cirrhosis and the usability as a marker in the diagnosis and follow-up.

Material and method: Patients were divided into 2 groups as decompensated and compensated according to their clinical stages. Detailed medical history, physical examination, laboratory and radiological assessments of the patients included in the study was obtained. One tube of blood was taken from each individuals in the patient and control groups and kept at -85 degrees Celsius. Markers were studied using the ELISA method.

Results: A total of 97 patients with cirrhosis and 51 healthy control patients were included in the study. Of the patients, 43 were compensated (44.3 %), and 54 were decompensated (55.7 %). The

average age of all patients with cirrhosis was 53 (19–89), the compensated cirrhosis group was 50 (19–85), the decompensated cirrhosis group was 56 (19–89), and in the control group was 44 (18–72) years. The level of urotensin-II was found to be statistically significantly lower in patients with compensated cirrhosis. And no significant difference was found in all patients and patients with decompensated cirrhosis compared to the control group.

Conclusions: The level of urotensin-II, were found to be lower in patients with compensated cirrhosis, indicating that urotensin-II may play a role in the pathogenesis of cirrhosis in the early stage. Further studies can be conducted on the role of urotensin-II in the pathogenesis of cirrhosis and on its use as a marker.

P-0776

Fragmented QRS and decompensated cirrhosis

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Background/Aim: It has been reported that the fragmented QRS (fQRS) is related to left ventricular systolic dysfunction and diastolic dysfunction. The aim of this study was to determine the frequency of fragmented QRS (fQRS) in patients with decompensated cirrhosis and to evaluate the relationship between the presence of fQRS and systolic dysfunction and diastolic dysfunction.

Methods: The study included consecutive 228 patients with decompensated cirrhosis. fQRS pattern was described as presence of RSR' manifested as existence of additional R wave and notching in either R or S waves in ECG recordings. Conventional echocardiography and tissue doppler echocardiography were performed in all patients

Results: The prevalence of fQRS was 28 % (64/228) in patients with decompensated cirrhosis. The patients with fQRS had worse diastolic functions in comparison to the patients without fQRS (IVRT, 97 ± 36 versus 72 ± 19 ms, $p < 0.01$; DT, 234 ± 71 versus 179 ± 73 , $p < 0.01$; E/A, 0.85 ± 0.42 versus 1.39 ± 0.75 , $p < 0.01$; Em, 8.4 ± 2.7 versus 12.1 ± 3.6 cm/s, $p < 0.01$; E/Em ratio, 11 ± 08 versus 8 ± 22 , $p < 0.01$). In addition, the patients with fQRS had worse systolic functions in comparison to the patients without fQRS (Sm, 8.1 ± 1.2 versus 11.1 ± 1.8 , $p < 0.01$).

Conclusions: This study showed that the relationship between the presence of fQRS and cardiac dysfunction in patients with decompensated cirrhosis. These data suggest that fQRS may represent a novel noninvasive marker for cardiac involvement in patients with decompensated cirrhosis and further studies will be needed to confirm these findings.

P-0777

Higher mortality during intensive care unit and associated factors in patients with liver cirrhosis

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Background: Outcomes during the intensive care unit (ICU) in patients with liver cirrhosis are not completely understood. This study evaluated the ICU mortality in patients with liver cirrhosis.

Methods: Among 1,250,300 ICU patients identified from reimbursement claims of Taiwan's National Health Insurance Research Database in 2006–2013, we conducted a nationwide retrospective cohort study of 57253 patients with liver cirrhosis. With propensity score-matching by sociodemographics, history of disease, and complications, 57253 ICU patients without liver cirrhosis were selected for comparison. Adjusted odds ratios (ORs) and 95 % confidence intervals (CIs) of ICU mortality were calculated in the multivariate logistic regressions.

Results: After matching and adjustment, ICU patients with liver cirrhosis had significantly higher mortality than those without liver cirrhosis (OR 1.76, 95 % CI 1.70–1.82). The association between liver cirrhosis and increased ICU mortality was significant in both sexes and every age group. The most significant ICU mortality was found in those with liver cancer (OR 2.02, 95 % CI 1.93–2.11), alcohol dependence syndrome (OR 2.24, 95 % CI 2.07–2.42), jaundice (OR 2.45, 95 % CI 2.29–2.61), ascites (OR 2.42, 95 % CI 2.32–2.52), gastrointestinal hemorrhage (OR 2.06, 95 % CI 1.97–2.16), and hepatic coma (OR 2.28, 95 % CI 2.18–2.39).

Conclusion: This nationwide propensity score-matched retrospective cohort study showed increased ICU mortality in patients with liver cirrhosis. Our findings suggest the urgency of preventing and managing liver cirrhosis by a multidisciplinary medical team for this specific population.

P-0778

Is microwave ablation a good alternative to partial splenic embolization to treat hypersplenism?

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Background: Hypersplenism has long been treated with splenectomy until partial splenic embolization (PSE) was adopted as a safer and less invasive alternative. However, PSE has been also associated with considerable side effects. Few preliminary studies introduced the loco-regional thermal ablation of a part of the spleen as a less invasive, safer and more effective technique. The aim of the current study was to compare the effectiveness and safety of microwave ablation (MWA) and PSE in the treatment of hypersplenism.

Patients and methods: Forty patients have been randomized into 2 groups; group I included 20 patients treated with percutaneous ultrasound guided MWA of the splenic parenchyma, and group II included 20 patients treated with transarterial PSE.

Results: Both groups showed a significant elevation of all blood elements after treatment. Comparison between the groups revealed that hemoglobin has significantly increased in group I after 3 months of follow up ($P = 0.022$), white blood cell count has significantly increased in group II ($P = 0.001$), while platelet count was higher in group II than group I at 1 month ($p = 0.001$); but this difference waned at 3 months ($P = 0.109$). Child Tourcoute Pugh (CTP) score increased significantly in group II than group I. This indicated a significant hepatic decompensation in group II only. Moreover, group II had a significant increase in morbidity and mortality.

Conclusion: Microwave ablation was effective and had a better safety than partial splenic embolization in the treatment of hypersplenism in patients with post-hepatitis C cirrhosis.

P-0779

Predictive upper gastrointestinal bleeding score for triaging patients-multicenter validation study

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Objectives: To determine the number of subjects with Upper Gastrointestinal Bleeding (variceal vs. non-variceal) that can be detected by the predictive upper gastrointestinal bleeding score (P-UGIBS) as confirmed by esophagogastroduodenoscopy (EGD). To determine association and significance of the independent variables in the P-UGIBS as compared with actual EGD findings.

Methods: Patients from four institutions who presented with UGIB from January 2010 to December 2012 were classified as variceal or non-variceal based on the P-UGIBS. EGD was compared with corresponding P-UGIBS and statistical analysis done. Inclusion criteria were presence of UGIB, EGD within 72, EGD finding with variceal or non-variceal bleeding and patients aged >18 years. Exclusion criteria were unidentified cause of UGIB, and subjects with no EGD done.

Results: Total of 594 patients presented with UGIB underwent EGD from January 2010 to December 2012. The Variceal group, 201 cases were predicted from a total of 206 with a risk ratio of 0.9758 (p-value 0.719). Binary logistic regression 97.6 %, Pseudo R squared 83.80 % (p value of 0.000). The Non variceal group, 251 cases were predicted from a total of 388 with risk ratio of 0.6774 (p-value 0.001). Binary Logistic Regression 64.70 %, Pseudo R squared 72.70 % (p value of 0.605).

Conclusion: The PUGIBS for variceal bleeding was able to detect majority of patients with variceal bleeding. Independent variables were significant predictors of the score. The PUGIBS for non-variceal bleeding was able to detect 64.70 % of cases and none of the variable were significant predictor of the scoring system.

P-0780

Alteration of serum lipid profile with severity of liver damage in cirrhotic patients

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Background: Cirrhosis is the end stage of any chronic liver disease. It is reasonable to expect an abnormal lipid profile in those with severe liver dysfunction. Lipid level can be used to determine the condition and estimate the prognosis of the patients. Due to the high prevalence of cirrhosis in Bangladesh this study was conducted to determine lipid profile and to assess if it relates to the severity of cirrhosis.

Method: An observational cross sectional study was conducted in Department of Hepatology, Bangabandhu Sheikh Mujib Medical University from Jan 2010 to Dec 2011. 100 patients (75 cirrhotics of 18–70 years of age group and 25 age and sex-matched disease control with normal BMI) were selected. The severity of liver cirrhosis was measured with Child-Pugh and MELD score. Fasting lipid profile was measured of each patient. Each component of serum lipid profile was correlated with each grade of severity of cirrhotic patients as well as control patients.

Results: Most of the cirrhotic patients were of 41–50 years age group. We found that serum TC, HDL, LDL in patients with cirrhosis is inversely correlated with severity of cirrhosis but there was no correlation between Child-Pugh score and TG. The mean MELD score had no significant correlation between any component of serum lipid profile (TC, HDL, LDL, TG) in decompensated cirrhosis.

Conclusion: There is a declining pattern of some parameters of serum lipid profile with severity of liver damage in cirrhosis.

P-0781

A/H1N1/09 influenza is associated with high mortality in liver cirrhosis

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Background: A/H1N1/09 influenza is known to be associated with a high risk of complications in patients with chronic diseases. Patients with cirrhosis are now being recognized as another high risk group for influenza morbidity and mortality.

Methods: We report a cluster of suspected A/H1N1/09 infection in 34 patients admitted to a hepatology ICU. The pattern of spread, clinical outcome, and respiratory parameters of A/H1N1/09 strains from a study group of 9 in-patients (who tested positive) were compared with those from a control group of 25 inpatients with influenza-like illness and with cirrhosis.

Results: A/H1N1/09 infection was confirmed in 9/34 (26.47 %) in-patients. Eight of 9 (88.8 %) patients with H1N1 and cirrhosis died of pneumonia and acute respiratory distress syndrome despite antiviral treatment. Ten of 25 (40 %) of the control group of cirrhotic patients without H1N1 died. Infections whether fungal or bacterial and portal hypertension are independent risk factors for disease severity in patients with cirrhosis. Vaccination, preventive and early antiviral treatment with oseltamivir and a strict control of nosocomial spread should be prioritized in patients with cirrhosis during epidemic influenza.

Conclusion: The current pandemic of the new H1N1 virus is progressing, and physicians and hepatologists should be aware of the potential effects of this infection on patients with chronic liver disease.

P-0782

Prophylaxis for esophageal varices can improve THE QOL in patients with PVTT.

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Objective: The aim of this study was to evaluate the effect of the prophylaxis for esophageal varices (EV) in advanced HCC with portal vein tumor thrombosis (PVTT).

Methods: A total of 118 consecutive patients were diagnosed as advanced HCC with PVTT in our hospital from April 2010 to March 2012. For one year before March 2011, hemostasis was achieved endoscopically in the event of variceal rupture (Group A). For one year after April 2011, we performed the prophylactic band ligation to EVs with over F2 and red color sign (Group B). The cumulative bleeding rate and overall survival were compared between 2 Groups.

Results: A total of the 118 patients (male: female, 94:24; mean age 67.2 yrs) were assigned to Group A (N = 63) and Group B (N = 55). 109 patients died and 13 patients vomited blood. The median survival times were 8.2 and 8.4 months, respectively (P = 0.95). Hematemesis was occurred: 10 (15.8 %) of Group A and 3 (5.5 %) of Group B (P = 0.071). The number of death related to hematemesis was significantly different: 6 of Group A and 0 of Group B (P = 0.0052). The number of the patients who needed the emergency endoscopy in the night was in 5 of Group A and 1 of Group B, respectively.

Conclusion: This study suggested that prophylactic treatment for EVs in patients with PVTT doesn't prolong the prognosis but can prevent the death of hematemesis. Prophylactic therapy may reduce the emergency endoscopy in the night and improve the QOL of both patients and medical staffs.

P-0783

Spleen stiffness alone vs. liver to spleen stiffness in the prediction of portal hypertension.

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Background and aims: We previously established a spleen stiffness measurement (SSM) of ≥ 19.45 kPa, and a liver to spleen stiffness (LSM:SSM) ratio using transient elastography were significant for detecting portal hypertension. The aim of our current study is to explore these findings in a larger group of cirrhotic patients. We also evaluated the clinical significance of individual SSM values vs. LSM:SSM ratio in providing higher sensitivity for PH.

Methods: 52 consecutive tests were conducted on cirrhotic patients (Mean age 60.9/ BMI 30.7) underwent both LSM and SSM; half of which had proven portal hypertension in the form varices, \pm Portal hypertensive gastropathy. Patients were divided by LSM values (< 20.00 , ≥ 20.00 kPa); and disease etiology.

Results: 100 % success rate was achieved. The mean SSM (Pr) in Supine and RLD position for all patients were 35.65 kPa (Pr = 0.988) and 27.64 kPa (Pr = 0.0003), respectively. Further break down is seen in Table 1.

Overall LSM:SSM ratio for all patients with LSM <20.00 kPa, ≥ 20.00 kPa were 0.46 and 0.82, respectively. Further ratio break-down is seen in Table 2. Using T-test analysis for LSM of 20.00 kPa, the best cut-off value for RLD-SSM equals 20.90 kPa, yielding a SN = 0.77, and SP = 0.80.

Conclusion: Individual SSM values vary depending on disease etiology & body position; whereas LSM:SSM ratio is less effected by outside variables. A spleen stiffness value ≥ 20.90 kPa and a LSM:SSM ratio of 0.82 are indicative of clinically significant portal hypertension, and therefore, of variceal formation. Further studies are needed to confirm these findings.

Table 1: Individual SSM values based on body position and disease etiology

Mean SSM Values for:	HBV	HCV	FLD	AH	OVERALL (kPa)
Supine (LSM <20.00kPa)	36.31	14.13	43.74	54.56	35.71
Supine (LSM ≥ 20.00 kPa)	34.40	26.79	31.01	51.73	35.60
Supine (ALL)	35.20	21.72	35.55	55.20	36.92, P=0.9887
RLD (LSM <20.00kPa)	19.19	15.28	11.08	12.82	14.80
RLD (LSM ≥ 20.00 kPa)	23.21	35.54	35.00	34.93	34.90
RLD (ALL)	25.87	27.02	27.00	30.05	27.64, P=0.0003
Total Number of Pts	12	18	14	11	55

Table 2: LSM:SSM ratios based on body position and disease etiology

LSM:SSM RATIOS	HBV	HCV	FLD	AH	ALL
Supine (LSM <20.00kPa)	0.46	0.39	0.42	0.35	0.41
Supine (LSM ≥ 20.00 kPa)	0.58	0.37	0.43	0.39	0.46
RLD (LSM <20.00kPa)	0.59	0.43	0.40	0.45	0.52
RLD (LSM ≥ 20.00 kPa)	0.24	0.79	0.77	0.78	0.82

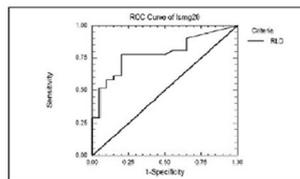


Figure 1: Specificity of SSM-RLD for EV in all patients with LSM ≥ 20.00 kPa.

P-0784

Pathological characteristics of spleen in liver cirrhosis and idiopathic portal hypertension

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Background: Spleen stiffness measured by ultrasonographic elastography is increased in patients with liver cirrhosis (LC) and idiopathic portal hypertension (IPH) than in normal controls. However, the pathological characteristics of spleen in LC and IPH patients is not yet clear. We tried to characterize the pathological features of spleen in LC and IPH patients.

Methods: We studied splenic tissues of 53 patients with HCV associated liver cirrhosis and 5 patients with IPH. These patients were underwent splenectomy in order to lower portal pressure. Nine cases without liver disease were used as normal control. We evaluated all splenic specimens, histologically (e.g. presence of passive congestion and Gamma-Gandy nodules, thickness of capsule and cord, number of trabeculae and lymph follicle, minor axis length of sinus).

Results: In LC patients, passive congestion was observed more patients than in IPH patients and controls (LC; 42 in 53 patients vs. IPH; one in 5 patients, control; none in 9 cases, $p < 0.01$). Minor axis length of sinus was longer in LC patients than in controls ($p < 0.01$). In IPH patients, the number of trabeculae was increased compared with LC patients ($p = 0.03$). Presence of Gamma-Gandy nodules, thickness of capsule, number of lymph follicle in LC and IPH patients were not significantly different from those in controls.

Conclusions: In liver cirrhosis, increase of splenic stiffness might be caused by passive splenic congestion with dilated splenic sinus. In IPH, it was suggested that spleen of IPH patients was hyperplasia.

P-0785

Fibroscan is superior to HVPG in assessing advanced hepatic fibrosis

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Aim: Aim of the study was to compare between Fibroscan and hepatic venous pressure gradient (HVPG) to assess extent of hepatic fibrosis in patients with chronic liver disease.

Methods: Eighteen patients diagnosed with cirrhosis of liver were included in the study. Diagnosis was made on the basis of clinical examination, laboratory reports and abdominal CT scan findings. Informed, written consent was obtained from all. There were 14/18 (87.8 %) males and 4/18 females (22.2 %). Fibroscan and HVPG were done in all patients to make a comparable assessment of their utility.

Results: On Fibroscan, 15/18 (83.3 %) patients had features of advanced hepatic fibrosis ($\geq F3$). On the otherhand, 11/18 (61.1 %) had portal hypertension at HVPG (≥ 7 mm of Hg), compatible with advanced hepatic fibrosis.

Conclusion: Fibroscan is widely accepted as a dependable way of assessing advanced hepatic fibrosis. The study suggests that it is also superior to HVPG in this regard. However study with larger sample size is needed to reach a definite conclusion. Also, it would be required to assess if these two procedures are equally applicable in mild and moderate fibrosis.

P-0786

Yearly variation in the portal hemodynamic parameters on Doppler sonography

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Background/aims: Portal hemodynamics may reflect the severity of portal hypertension, which is informative in the management of the patients. However, the variation in the portal hemodynamic parameters during the natural course has yet been determined. The aim of the study was to determine the variation of portal hemodynamics on Doppler sonogram over one-year interval in patients with portal hypertension.

Methods: This prospective study consisted of 65 patients with portal hypertension (cirrhosis 43, non-cirrhotic portal hypertension 22; male 36, female 29; age 38–78 years old, mean \pm standard deviation 60.9 ± 9.6). All subjects underwent Doppler ultrasound twice or more over one-year interval. Portal hemodynamic parameters including diameter, flow direction, velocity and flow volume were assessed by pulsed Doppler in the portal trunk and the splenic vein. Variation index (VI) was determined by the percentage ratio of the absolute difference between the two-time of measurement data divided by the baseline data.

Results: The VI of the diameter, flow velocity and flow volume was 8.9 and 11.2, 20.1 and 22.8, and 28.8 and 38.0 % in the portal trunk, and 14.5 and 17.6, 29.7 and 22.7, and 36.5 and 44.1 % in the splenic vein, in cirrhosis and non-cirrhosis respectively. There was no significant difference with the VI and intra-operator variability with development collateral vessels, and baseline clinical findings between cirrhosis and non-cirrhosis patients.

Conclusions: The data may be applicable as a reference value when the time-related changes of portal blood flow need to be evaluated.

P-0787

Esophageal varices and platelet sequestration, Effect of variceal eradication on platelet count

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Background: In Egypt, viral hepatitis along with infection with *Schistosoma mansoni* are the major causes of chronic liver disease. variceal bleeding is the most frequent and severe consequence of portal hypertension in patients with cirrhosis.

Abnormalities in hematological parameters are common in patients with cirrhosis due to portal hypertension-induced sequestration, alterations in bone marrow stimulating factors, viral- and toxin-induced bone marrow suppression. The aim of this work was to study the effect of variceal eradication either by Endoscopic band ligation or injection sclerotherapy on platelets count among a group of Egyptian cirrhotic patients presented with bleeding oesophageal varices.

Methods: This study was conducted from Jan 2011 to Aug 2011 on 43 patients with liver cirrhosis, child group A and B, with esophageal varices presented by hematemesis or had endoscopic feature indicating either band ligation or injection sclerotherapy. Patients were divided into 2 groups. Group 1 (22 patients) undergone band ligation and, group 2 (21 patients) undergone injection sclerotherapy. Ten liver cirrhosis patients Child group A, and B on 40 mg propranolol/day were the control group. All patients were followed for 3 month.

Results: Comparing Platelet counts at the end of the first and third month, for each group, in relation to the initial reading, There was a significant elevation in mean platelet count in the third month (159.00) compared to initial reading (122.17) in The control group (on B-blocker) $P = 0.024$, while Platelet count showed no significant changes all over the follow up period in both groups (patients undergone band ligation and injection sclerotherapy)

P-0788

Correlation of platelets count with endoscopic findings in Egyptian patients with liver cirrhosis

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Background: Portal hypertension is a common complication of liver cirrhosis that can lead to development of esophageal varices (OV) and PHG. Screening endoscopy had been recommended for early detection of OV and PHG in cirrhotic patients with portal hypertension. However this approach is limited by its invasiveness.

Objective: platelet count can predict the presence of (EV) in a cohort of Egyptian patients with liver cirrhosis.

Results: A total of 110 patients were included. The mean age was 54.39 ± 7.46 years; 73 (66.36 %) were men and 37 (33.64 %) were women. The etiology of cirrhosis included HCV in 107 of patients (97.27 %), hepatitis B in only 1 patient (0.9 %), cryptogenic in two (1.8 %). EV were present in 79.1 % (87 patients). as regard to thrombocytopenia, patients with platelet below 50,000: 30 % had no varices, 12.5 % had EV grade I, 20 % had EV grade II, and 37.5 % had EV grade III or IV. In patients with platelet between 50,000–100,000: 10.53 % had no EV, 36.84 % had grade I EV, 26.31 % had grade II EV and 28.21 % had grade III or IV EV. While in patients whom platelet was above 150,000: 37.5 % had no varices, 34.38 % had grade I EV, 18.75 % had grade II EV and only 9.37 % had grade III or IV EV.

Conclusion: It is better to maintain the strategy of upper gastrointestinal endoscopy for all patients with cirrhosis. However, Presence of large EV in patients with normal platelet count is less common.

P-0789

Visualization of the vascular architecture using SMI reflects the fibrosis in patients with HCV

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Background: Superb microvascular imaging (SMI) is an innovative Doppler ultrasound technology that employs a unique algorithm, allowing visualization of minute vessels with slow velocity. In the present study, we identified the hepatic vascular architecture of patients with hepatitis C virus (HCV) using SMI, and investigated the use of SMI in the evaluation of liver fibrosis.

Materials and methods: SMI was performed in 69 patients with HCV. The vascular architecture of the anterior-inferior portal vein was evaluated independently by two investigators on the monochrome mode of SMI on the same day as a liver biopsy procedure. The biopsy specimens were reviewed by a blinded pathologist using the METAVIR criteria. The SMI images were analyzed for vascularity by integrating the number of blood vessels over the imaged area (vascular density: VD).

Results: The VD for each stage of fibrosis were: 2.13 ± 0.61 in F0 ($n = 17$), 2.56 ± 1.08 in F1 ($n = 25$), 3.22 ± 1.36 in F2 ($n = 7$), 5.21 ± 1.41 in F3 ($n = 9$), and 8.17 ± 1.21 in F4 ($n = 11$). A steady stepwise increase in the VD was correlated with the stage of liver fibrosis ($p < 0.0001$). The diagnostic accuracy of the VD in the prediction of F4 was 0.941 (95 % CI: 0.853–0.974, $p < 0.0001$). The cut-off values of the VD for the prediction of F4 were 6.21 with 93.8 % sensitivity and 83.7 % specificity. **CONCLUSIONS:** Noninvasive assessment of VD with SMI appears to be a reliable tool for detecting significant fibrosis or cirrhosis in patients with HCV.

P-0790

Comparison between HVPG and endoscopy of UGIT in assessing severity of portal HTN in cirrhotics

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Aim: Aim of the study was to compare the relative utility of hepatic venous pressure gradient (HVPG) and endoscopy of upper gastrointestinal tract (UGIT) for the assessment of severity of portal hypertension in liver cirrhosis patients.

Methods: Forty five patients diagnosed with cirrhosis of liver were included in the study. Informed, written consent was obtained from all. There were 33/45 (73.3 %) males and 12/45 females (26.7 %). Four patients had past history of gastrointestinal (GI) bleeding.

Results: On endoscopy of UGIT, 20/45 (44.4 %) patients had features of portal hypertension. Of them, 14/20(70 %) had esophageal varices (EV), 2/20(10 %) had portal hypertensive gastropathy (PGH) and 4/20(20 %) had both EV and PHG. On the contrary, portal hypertension was diagnosed in 32/45(71.1 %) patients at HVPG (≥ 7 mm of Hg). EV needing therapeutic intervention (F2 and F3) was found in 10/45(22.2 %) patients at endoscopy of UGIT compared to 14/45 (31.1 %) at HVPG (≥ 13 mm of Hg). Of the 4 patients with past history of GI bleeding, 3 had F3 EV, but on HVPG all had significant portal hypertension.

Conclusion: Portal hypertension is not only an important cause of upper GI bleeding in cirrhosis, but also contributes significantly to the development of ascites. The study demonstrates a comparable better implication of HVPG over endoscopy of UGIT for assessment of portal hypertension in liver cirrhosis. This may aid profoundly in the long term management and prognosis of these patients.

P-0791

Pathogenesis of portal hypertension and the intrahepatic hemodynamics in liver cirrhosis

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Background and aims: It is very important to reveal the pathogenesis of portal hypertension in patients with liver cirrhosis. We evaluated the arrival time of Sonazoid to intrahepatic vessels by using contrast enhanced ultrasonography to reveal the pathogenesis of portal hypertension in patients with liver cirrhosis.

Methods: Thirteen healthy volunteers, eighteen chronic hepatitis and thirty-eight patients with liver cirrhosis were enrolled in this study. First, we evaluated the arrival time to intrahepatic vessels, hepatic artery(HA), portal vein(PV), hepatic vein(HV), after intravenous administration of Sonazoid (0.5 ml/body). Secondly, shear wave ultrasound elastography of the liver and spleen was performed on 28 patients using Virtual Touch Quantification (VTQ) by Siemens ACUSON S2000/S3000. This study was approved by the institutional ethics committee.

Results: First, in patients with liver cirrhosis, arrival time to HA and HV was significantly shorter than those in healthy volunteers ($P < 0.01$). and the differences between HA and HV, PV and HV in patients with liver cirrhosis were significantly smaller than those in healthy volunteers ($P < 0.01$). Among the patients with liver cirrhosis, the arrival time to HA, PV and HV in patients with esophageal and gastric varices (EGVs) was significantly shorter than that in patients without EGVs ($P < 0.05$). Secondly, Vs values in the liver was significant correlation between the differences between HA and HV, HA and PV.

Conclusion: In patients with liver cirrhosis, we observed shorter arrival time to intrahepatic vessels. Our data suggested that intrahepatic shunt plays an important role in the pathogenesis of portal hypertension.

P-0792

Both elevation of HVPG and LS can predict mortality in non-critically ill patients with cirrhosis

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Purpose: The pattern of elevation of hepatic venous pressure gradient (HVPG) and liver stiffness (LS) in predicting long-term mortality in non-critically ill patients with cirrhosis is not yet studied.

Materials and methods: We retrospectively and consecutively collected data on the enrolled 105 non-critically ill patients with cirrhosis whose HVPG and LS by shear wave elastography were measured concurrently between December 2009 and September 2013. Total patients were categorized into four groups according to the elevation pattern of HVPG and of LS at the cut-off value of 16 mmHg and 25 kPa that were determined by our previous study, respectively.

Results: Among 105 patients, 32 were in group 1 (both low HVPG and LS), 18 in group 2 (high HVPG and low LS), 21 in group 3 (low HVPG and high LS), and 34 in group 4 (both high HVPG and LS). During median follow-up period (18 months), 15 patients died, and among them 11 were in group 4. The cumulative survival rate of group 4 was much lower than the other groups ($P = 0.006$). In univariate analysis for death, factors associated with long-term mortality were serum sodium ($P = 0.004$), refractory ascites ($P = 0.012$), LS ($P = 0.013$), HVPG ($P = 0.045$) and group 4 ($P = 0.012$). In multivariate analysis, only two factors of group 4 (HR 5.42, $P = 0.011$)

and high LS (HR 1.06, $P = 0.049$) were positively correlated with long-term mortality.

Conclusion: Concurrent elevation of HVP and LS showed the highest long-term mortality risk in non-critically ill patients with cirrhosis.

P-0793

Enhanced VASH1 expression in splenic sinus lining cells relates to splenomegaly in IPH

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Aims: Vasohibin-1 (VASH1) is dominantly expressed in endothelial cells, induced by the stimulation with VEGF or FGF-2. A recent study has shown that VASH1 regulates portal hypertension-associated pathological angiogenesis in the liver, and VASH1 is a potential target of therapy for portal hypertension and cirrhosis (Coch L et al. Hepatology 2014). This study was performed to examine histological features of spleen of idiopathic portal hypertension (IPH) in relation to the expression of angiogenesis associated molecules including VASH1.

Methods: Paraffin-embedded tissue sections of spleen from patients with IPH, chronic viral hepatitis/liver cirrhosis (CVH/LC) and normal subjects were used. Immunohistochemical analysis was performed for the expression of VASH1, VEGF, FGF-2 and PCNA. The staining results were semi-quantitatively evaluated. Serum levels of VEGF and FGF-2 were measured using ELISA for patients with IPH, CVH/LC and healthy controls.

Results: In splenic sinus lining cells, the immunohistochemical expression of VASH1 and FGF-2, and the PCNA-labeling index were significantly increased in IPH compared to those in CVH/LC and normal groups. The VASH1 expression was positively correlated with the FGF-2 expression and the PCNA-labeling index. The immunohistochemical expression of VEGF was faint or invisible in all experimental groups. Serum levels of FGF-2 tended to be elevated in IPH and CVH/LC groups compared to those in healthy controls. Elevation of serum VEGF levels was not observed in IPH, whereas they were significantly elevated in CVH/LC. **Conclusions:** Splenomegaly of IPH was associated with active proliferation of sinus lining cells accompanied by the enhanced expression of VASH1 and FGF-2.

P-0794

Clinical characteristics and outcome of Budd-Chiari Syndrome at a tertiary care hospital in Pakistan

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Introduction: Budd-Chiari syndrome (BCS) is defined as hepatic venous outflow tract obstruction, which could be at any level from the hepatic veins up to the entrance of the IVC at the right atrium. **Objectives:** To determine the clinical characteristics of Budd-Chiari syndrome, outcome and causes at a tertiary care hospital, in Karachi.

Results: 45 patients' charts were reviewed; 26 (57.8 %) were of male patients. Primary BCS was seen in a total number of 27 (60.0 %) patients. The median (interquartile range) age at diagnosis was 26.0 (20.5 to 34.5) years. In symptomatology, abdominal pain was reported in 25 (55.6 %) patients and abdominal distension in 36 (80.0 %) patients. 37 (82.2 %) patients had ascites and 14 (31.1 %) had hepatomegaly. Using imaging techniques, such as Doppler US, a combined HV/IVC block was reported in 25 (55.6 %) patients. The underlying cause was a protein C and protein S deficiency in 22 (78.6 %) patients and 17 (60.7 %) patients, respectively, out of the 28 patients who were tested. Antithrombin III deficiency was detected in 14 (58.3 %) of the 24 tested patients. For management, anticoagulants were given to 24 (53.3 %) patients. TIPS was done in 11 (24.4 %) of the patients. Death was the outcome in 3 (6.7 %) patients.

Conclusion: This study has thrown light on the clinical characteristics of BCS which will help direct further research on the management of BCS in Pakistan.

P-0795

Endoscopic ligation and beta blocker versus monotherapy in primary prophylaxis of variceal bleeding

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Background: Prevention of variceal bleeding is one of the most important strategies to reduce mortality in portal hypertensive patients.

Objectives: To determine whether the combination of endoscopic ligation and beta blocker is more effective than endoscopic band ligation or beta blocker alone in the primary prophylaxis of esophageal variceal bleeding.

Methodology: Literature search was performed on GastroHep, Highwire Press, Cochrane database, Google scholar, Medline and Pubmed database for the National Library of Medicine. Randomized controlled trials comparing endoscopic ligation and beta blocker with endoscopic band ligation or beta blocker alone were investigated. Main outcomes were variceal bleeding, all-cause mortality, recurrence of varices and adverse events. Statistical analysis was done using Review Manager 5.3 with odds ratio as effect measure.

Results: Three randomized controlled trials with a total of 176 patients were analyzed. No significance difference was noted in the occurrence of first variceal bleed (Odds ratio (OR) 0.93; 95 % confidence interval (CI) [0.45–0.93]; $p = 0.85$) and all-cause mortality (OR 0.99; 95 % CI [0.54–1.81]; $p = 0.97$) between treatment groups. Recurrence of esophageal varices was found to be lower in the combination group compared to monotherapy (OR 0.24; 95 % CI [0.10–0.59]; $p = 0.002$); however, resulted in significantly higher incidence of adverse events (OR 1.97; 95 % CI 1.09–3.54; $p = 0.02$).

Conclusion: Albeit combination therapy decreased the recurrence of esophageal varices, current studies does not support its use in the primary prophylaxis of esophageal varices. Occurrence of variceal bleeding and mortality were not altered and adverse events were noted to be higher with combination therapy.

P-0796

Diagnostic accuracy of spleen stiffness for the portal hypertension in patients with liver cirrhosis**Yoshitaka Takuma^{1,2}, Youichi Morimoto², Hiroyuki Takabatake², Hiroshi Yamamoto²**¹Department of Internal Medicine, Hiroshima City Hospital, Hiroshima, Japan; ²Department of Gastroenterology, Kurashiki Central Hospital, Kurashiki, Japan**Purpose:** To evaluate diagnostic accuracy of spleen stiffness (SS) and liver stiffness (LS) values measured by acoustic radiation force impulse (ARFI) imaging for evaluating portal hypertension in patients with liver cirrhosis.**Materials and methods:** Institutional review board approval and informed consent were obtained, and the prospective study was performed at a single center. From February 2012 to August 2013, 60 liver cirrhotic patients (mean age, 70.8 years; 34 men, 26 women) with measurements of hepatic venous pressure gradient (HVPG), LS, SS, gastrointestinal endoscopy, and laboratory data were included.**Results:** The efficacy of the parameters for the evaluation of portal hypertension was analyzed using Spearman's rank-order correlation coefficient and receiver operating characteristic (ROC) curve analysis. The correlation coefficient between SS and HVPG ($r = 0.876$) was significantly better than that between LS and HVPG ($r = 0.609$, $P < 0.0001$). The area under the ROC (AUROC) values of SS for the identification of clinically significant portal hypertension (CSPH; HVPG ≥ 10 mmHg), severe portal hypertension (SPH; HVPG ≥ 12 mmHg), esophageal varices (EV), and high-risk EV were significantly higher (0.943, 0.963, 0.937 and 0.955, respectively) than those of LS, spleen diameter, platelet count, and platelet count/spleen diameter ratio (all $P < 0.05$). SS was able to accurately rule out the presence of CSPH, SPH, EV, and high-risk EV (negative likelihood ratio 0.051, 0.056, 0.054, and 0.074, respectively).**Conclusion:** SS is reliable and has better diagnostic performance than LS for identifying portal hypertension in liver cirrhosis.

P-0797

Efficacy and safety of danaparoid sodium followed by warfarin or edoxaban for treatment of PVT**Yuko Nagaoki, Hiroshi Aikata, Norihito Nakano, Humi Honda, Yuki Nakamura, Masahiro Hatoooka, Kei Morio, Tomoki Kobayashi, Takayuki Hukuhara, Tomokazu Kawaoka, Masataka Tsuge, Akira Hiramatsu, Michio Imamura, Yoshiiku Kawakami, Kazuaki Chayama**

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Aim: To assess the efficacy and safety of danaparoid sodium followed by warfarin or edoxaban for treatment of portal vein thrombosis (PVT) with liver cirrhosis.**Methods:** A consecutive 42 cirrhotic patients with PVT were enrolled in retrospective cohort study. Oral anticoagulants have been treated following initial danaparoid sodium for 2 weeks of followed by the evaluation of PVT reduction and adverse events. PVT volume was

measured by dynamic CT before treatment, after 2 weeks and 6 months.

Results: Child-Pugh grade A, B and C were noted in 25, 12 and 5 patients, respectively. Twelve patients received edoxaban and 30 received warfarin, significant differences in characteristics factors did not recognized. The median volume of PVT before treatment, after two weeks and 6 months were 3.6, 1.3 cm³ ($P < 0.001$), 2.5 cm³ ($P = 0.011$), respectively and is a significant reduction effect compared to before treatment. After 6 months, as independent successful factors for PVT reduction, the only factor with successful influence was edoxaban therapy ($P = 0.045$ HR 6.720). The change of PVT volume after 6 months, showed significantly reduced effect in edoxaban group (1.10 cm³) than warfarin group (2.66 cm³) ($P = 0.048$). The primary safety outcome of clinically relevant bleeding occurred in 3 of 12 (25 %) patients in edoxaban group and 2 of 30 (7 %) in warfarin group.**Conclusion:** Danaparoid sodium for the treatment of PVT in patients with liver cirrhosis was safe and effective. Furthermore, edoxaban is an effective and could have potential as one of the treatment options in PVT accompanied by cirrhosis.

P-0798

EVL following variceal rupture in patients with hepatocellular carcinoma and portal vein thrombosis**Toshihiro Kawai, Yoko Yashima, Takafumi Sugimoto, Takahisa Sato, Miho Kanda, Shinpei Sato, Shuntaro Obi**

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Introduction: The outcome of ruptured variceal treatment in patients with hepatocellular carcinoma (HCC) and portal vein tumor thrombus (PVTT) is unclear. We evaluated the outcomes defined as rebleeding, of patients with PVTT who underwent emergency treatment of potentially fatal variceal bleeding.**Methods:** A total of 62 consecutive patients with PVTT and endoscopically proven esophageal or gastric variceal bleeding, who were admitted in our hospital between 2007 and 2012, were included. Emergent endoscopy was performed within 24 h after hemorrhage. The bleeding varices were ligated using a pneumatic EVL device. Rescue therapies (i.e., Sengstaken-Blakemore balloon tamponade) were applied when necessary.**Results:** Most of patients were decompensated cirrhosis with the liver function stages of the patients were Child-Pugh B (56 %) or C (36 %). 58 patients (94 %) had esophageal variceal bleeding and 4 (6 %) patient had gastric variceal bleeding. 35 patients (56 %) had portal vein tumor thrombosis of main trunk. Except for one patient, the bleeding was managed using endoscopic variceal ligation (98 %). 24 patients experienced rebleeding, and a median overall survival time of 36 days. Absence of portal vein tumor thrombus in the main trunk was associated with rebleeding-free survival (hazard ratio 3.706, $p = 0.0223$), and α -fetoprotein-L₃ < 37.4 % (hazard ratio 0.464, $p = 0.015$) and Child-Pugh Class A/B (hazard ratio 0.398, $p = 0.007$) were related to over-all survival.**Conclusion:** EVL is a safe and effective treatment of variceal ruptures, even in patients with HCC and PVTT. After successful hemostasis, alleviation of the underlying liver function impairment and tumor control are equally important for better prognosis.

P-0799

VBT: a new test of variceal source among patients presenting with upper GI bleeding**Tahiri Joutei Hassani Mohammed, Hliwa Wafaa, Hadad Fouad, Bellabah Ahmed, Badre Wafaa**

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Upper gastrointestinal bleeding (UGIB) is life-threatening emergency. Early diagnosis of the variceal or non-variceal source of bleeding can orient pharmacological treatment before endoscopy.

Aim: To identify clinical parameters before endoscopic evaluation that would be predictive of a variceal origin of UGIB, develop and validate a score to distinguish variceal from non variceal etiologies of UGIB.

Methods: A retrospective study was conducted in tertiary gastroenterology center over the period from January 2013 through APRIL 2015. Data regarding demographics, including historical, physical examination, initial laboratory investigations, endoscopic findings. Statistical analysis was carried out by logistic regression modelling, and receiver-operating characteristic curves

Results : Of the 100 patients with UGIB 52 % had variceal and 48 % non-variceal bleeding. Three factors were associated with variceal UGIB : prothrombin time, ALT and Total bilirubin We developed a new prediction score, named the Variceal bleeding Test :VB TEST, using a regression equation composed of this panel of biomarkers. VB-Test could differentiate variceal from non variceal etiologies The VB test score allowed the correct identification of patients with variceal etiologies of UGIB with an area under the receiver operating characteristic curve of 0.91 (95 % CI 0.85–0.97). Positive predictive value (PPV) and negative predictive value (NPV) were: 85.7 and 91.8 %, respectively. VB Test was validated prospectively in another group Of 129 patients: PPV and NPV were 82 and 89 %, respectively.

Conclusion: VB TEST could differentiate variceal from non variceal etiologies of UGIB with good accuracy; this finding is useful to carry out pharmacological therapy before endoscopy.

P-0800

Audit of the practice of elective endoscopic variceal ligation and its complication in Singapore**PY Loh, TL Ang, Jessica Tan, KM Fock, EK Teo**

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Variceal bleeding can be life threatening with associated mortality of 20 % at 6 weeks. Guideline suggests that in patient with medium/large varices and no previous bleeding (primary prevention), non selective beta blockers (NSBB) or EVL is recommended whereas in patient with previous bleeding (secondary prevention), combination of NSBB plus EVL is recommended. However, EVL is associated with the risk of post banding bleeding of 2.74–6.7 % with Schepke et al showed 2.6 % of mortality from bleeding after EVL.

Methods: Retrospective study from 1st Jan 2010 to 31st Dec 2013, patients were identified from EVL procedure code and had it done as an elective procedure.

Results: 107 patients underwent 248 elective EVL over 4 years. 61.2 % of the patients had previous history of variceal bleeding.

92.5 % of patients in primary prevention and 98.4 % of patients in secondary prevention were on combination treatment (EVL + NSBB).The incidence of bleeding was 1.6 % (4 episodes). The mean delay of bleeding was 4.5 days (range 1–13). No mortality was caused by post banding bleeding.Subgroup analysis comparing non-bleeding and bleeding EVL episodes showed larger size of varices ($p = 0.01$) and concomitant presence of gastric varices ($p = 0.004$) were associated with higher risk of post banding bleeding. No significant association between Child Pugh status, thrombocytopenia, coagulopathy and bleeding.

Conclusions: Large proportion of patients who had EVL for primary prevention were given beta blocker unnecessarily. This will subject them to the side effects of beta blocker without added benefit. The risk of bleeding and mortality is low after elective EVL with certain features can predict the bleeding risk.

P-0801

Statins reduce esophageal variceal rebleeding in post-endoscopic variceal ligation patients**Sien-Sing Yang^{1,2}, Yi-Wen Huang^{1,3,4}, Szu-Chieh Fu¹, Sien-Sing Yang^{1,2}, Ting-Chuan Wang⁵, Jui-Ting Hu^{1,2}, Ding-Shinn Chen⁴**

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Background: Statins reduce fibrosis progression and portal hypertension. We aimed to evaluate the effect of statins to prevent variceal rebleeding in post endoscopic variceal ligation (EVL) hepatitis B patients.

Methods: A nationwide cohort study was conducted by Taiwanese National Health Insurance Research Database. We identified 3052 hepatitis B patients with previous EVL from years 1997–2009. The statins cohort and 1:5 ratio propensity score matched non-statin cohort were followed-up from the index date of first EVL until development of variceal rebleeding, withdrawal from insurance, or December 2009.

Results: The modified Kaplan-Meier survival analysis and Gray methods were used to adjust the competing risk data ratios which showed that a significantly lower cumulative incidences of variceal rebleeding ($P < 0.01$) among post-EVL patients under statins as compared to those without. The adjusted hazard ratio (AHR) of statins user among post-EVL hepatitis B patients in the Cox proportional hazard analysis for variceal rebleeding was 0.22 (95 % CI, 0.07–0.73, $P = 0.01$) after controlling for differences in age, gender, propranolol or nadolol, subsequent EVL, CHB treatment, hepatocellular carcinoma, ascites, hepatic coma, non-statin lipid-lowering drugs, and triglyceride lipid-lowering drugs.

Conclusions: Statins independently prevented variceal rebleeding in high risk post-EVL hepatitis B patients in this retrospective study. This protective effect was greater with longer duration of statins use. Further prospective studies need to be performed to evaluate the results of this study.

P-0802

To determine the frequency of upper GI bleeding and its predicting factors in CLD**Muhammad Adnan¹, Jahangir Liaquat², Muhammad Akbar¹**¹Department of Internal Medicine, Isra University Hospital, Hyderabad, Sindh, Pakistan; ²Liaquat University Medical, Hyderabad, Sindh, Pakistan**Objective:** To determine the frequency of upper GI bleeding and its predicting factors and esophageal varices in the patients with liver cirrhosis disease admitted at medicine ward of Isra university hospital. Design Prospective and observational study.**Setting:** Isra University Hospital. Period March 2012 to August 2012 six months.**Methods:** Containing 100 patients, mean age was 45.8, and all the patients with cirrhosis disease were included in this study with liver cirrhosis disease. All patients were under went endoscopy and Frequency of upper GI bleeding and varices presentation and classification according to grade were noted.**Results** All the 100 patients were selected on the basis of presenting liver cirrhosis disease. Male were more found than the female with the mean age 45.8. Mostly cirrhotic patients were found with HCV positive and upper GI bleeding were noted in (40 %) of the cases. With the endoscopic finding mostly patients were noted in II and III grade of esophageal varices and according to child pug classification majority of patients was noted in class C, In addition, thrombocytopenia and red wale markings along with the presence of large sized varices were associated with the presence of esophageal varices.**Conclusions:** In the conclusion of this study we found majority of the cirrhotic patients with HCV, Esophageal varices and thrombocytopenia are the important factors of upper GI bleeding. Knowledge and etiology of this manuscript may helpful in the prevention of oesophageal varices and upper GI bleeding.

P-0803

Treatments of bleeding portal hypertensive duodenopathy**Takahiro Sato, Sho Kitagawa, Mutsuimi Kimura**

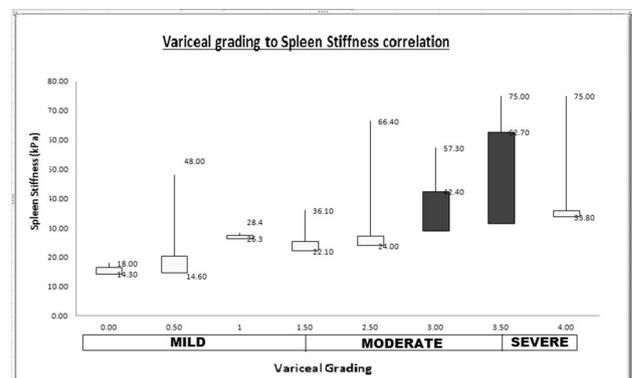
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Aim is to investigate the treatments of bleeding portal hypertension duodenopathy (PHD). Twenty-six patients with bleeding PHD were investigated. Endoscopic findings of bleeding PHD were investigated, and evaluated the therapeutic strategy. Twenty-six portal hypertensive patients had endoscopic bleeding duodenopathy lesions including duodenal angioectasia in 21 and duodenal varices in 5. The location of the bleeding duodenal angioectasia was the duodenal bulb in 3, descending portion in 18. Endoscopic findings of duodenal angioectasia were classified as follows: punctulate erythema (<1 mm) with oozing, and patchy erythema (a few mm) with oozing and, punctulate erythema in 8 and patchy erythema in 13. Eleven of 21 cases were followed-up with endoscopic observations and no further episodes of bleeding experienced in all 11. Endoscopic treatments was successfully performed for 10 of 21 cases of bleeding duodenal angioectasia; argon plasma coagulation (APC) in 8, APC plus clipping in 1 and endoscopic band ligation in 1. There were no further episodes of bleeding in 9 of 10 cases after the treatments. On the other hand, the location of the bleeding duodenal varices was the duodenal bulb in 1,

descending portion in 3 and third portion in 1. Treatments with interventional radiology and endoscopic procedures were successfully performed for all 5 duodenal varices: endoscopic injection sclerotherapy (EIS) using N-butyl-2-cyanoacrylate in 2, EIS plus balloon-occluded retrograde transvenous obliteration in 2, percutaneous transhepatic obliteration in 1.

Conclusions: Endoscopic treatment is useful for bleeding duodenal angioectasia. Interventional radiology and endoscopic procedures were effective for duodenal variceal bleeding.

P-0804

Comorbidity of diabetes mellitus and bleeding esophageal varices -short term outcome**Medhat A. Abdel Motelleb¹, Mustafa A. Al sayed², Kaled M. Metwally¹, Hassan A. Al shenawy¹**¹Hepatology department, National Liver Institute, Menoufia University, Shebeen al koom, Egpy; ²Internal Medicine department Al mansoural Hospital, al mansoura, Egypt**Background and aim:** information about comorbidity of bleeding esophageal varices in cirrhotics with diabetes mellitus is lacking, so we conduct our study to evaluate the impact of diabetes on the short term clinical outcomes of cirrhotic patients with bleeding esophageal varices and whether this could impact future plane of management.**Patients and methods:** A total of 435 cirrhotic patients were treated by endoscopic hemostasis on admission for acute GIT bleeding. After assessment of diabetes mellitus and presence of esophageal varices. Finally 200 patients with bleeding esophageal varices were divided into two equal groups based on presence or absence of diabetes mellitus. They were evaluated to verify the risk factors related to rebleeding, length of hospital stay and morbidity in both groups within 90 days follow up.**Results:** Diabetic group had higher rate of rebleeding (2.01 ± 1.42 vs. 1.46 ± 0.95 , $p < 0.001$), prolonged hospital stay (4.37 ± 1.86 vs. 3.38 ± 1.37 days $p < 0.05$) and higher in-hospital mortality within 30 days (19 vs. 3 %, $p < 0.001$) than non-diabetic group. Severity of liver disease reflected by the class of Child Pugh score had no impact on rebleeding in diabetic group. By univariate and multivariate analysis, and studying the area under the curve, values of INR in diabetic group above 1.76 were a risk factor for rebleeding.**Conclusion:** Diabetes mellitus is associated with higher rebleeding, prolonged hospital stay and higher in hospital mortality within 90 days in cirrhotic patients with acute bleeding esophageal varices.

P-0805

Hepatic hemodynamics and liver function before and after endoscopic injection sclerotherapy

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Background and aims: Endoscopic injection sclerotherapy (EIS) has been performed as treatment of esophagogastric varices (EGV) in Japan. We have previously reported it was useful to measure hepatic tissue blood flow (HTBF) by xenon computed tomography (Xe-CT) before and after EIS (J Gastroenterol. 2013). Our aim is to determine the suitable cases for whom it is suitable to obliterate portosystemic shunts.

Methods: The 45 liver cirrhotic patients who were treated with EIS and were performed Xe-CT. Etiology; HCV/HBV/NBNC/alcohol/PBC/AIH; 17/2/5/18/1/2. The portal venous TBF (PVTBF) and hepatic arterial TBF (HATBF) (ml/100 ml/min) were calculated before and 1 week after EIS. We defined delta-PVTBF which is subtraction PVTBF before EIS from PVTBF after EIS and divided into 2 groups. The plus delta-PVTBF was increased group (IG) and minus delta-PVTBF was non-increased group (NIG). We compared background and chronological change of liver function between IG and NIG.

Results: Elderly (over 65) and presence of paraesophageal veins (Para-v) were independent predictors of non-increased PVTBF after EIS by multivariate analysis. We confirmed that liver function improved after EIS in IG.

Discussion: The causes of non-increased PVTBF after EIS are receptive capacity of increased portal blood flow in the liver and presence of roots as to decrease the portal pressure. We considered that increased PVTBF led to improve liver function because we confirmed that liver function improved after EIS in IG.

Conclusion: We conclude that obliteration of EGV is a good indication for under 65 year-old patients without Para-v.

P-0806

Spleen stiffness: the missing link in detection of clinically significant portal hypertension

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Background and aims: Varices are a common feature of cirrhosis, and their prevalence parallels the severity of portal hypertension and liver dysfunction (1). This study aims to assess the use of spleen

stiffness measurement using transient elastography in predicting varices in a heterogeneous population of cirrhotic patients.

Methods: 47 consecutive cirrhotic patients were underwent both liver (LSM) and spleen stiffness measurements (SSM). 31 of the 47 patients had upper endoscopy to assess for the presence of varices.

Results: Patients had a mean age of 60.9 years, BMI of 30.7, where 61 % & 39 % were males and females, respectively. Mean LSM of all patients = 24.51 kPa, while mean SSM = 35.74 kPa. Using a T-test analysis for LSM of 20.00 kPa (2), the best cut-off value for SSM is equivalent to 20.90 kPa. A SSM \geq 20.90 kPa had a Sp = 1.00, Sn = 0.90, PPV = 0.87 in relation to presence of varices. Using a one-way Anova test, a P = 0.000 was seen for patients $<$ 20.90 kPa compared to $>$ 20.90 kPa. No statistical significance (P = 0.933) was seen within group for patients with spleen stiffness $>$ 20.90 kPa.

Conclusion: In our study, a liver stiffness value \geq 20.00 kPa, was inconsistent in predicting varices; where as a spleen stiffness \geq 20.90 kPa had a 100 % sensitivity for presence of varices. Spleen stiffness in turn, will enable clinicians timely non-invasive screening and intervention for varices. A larger study assessing spleen stiffness based on disease etiology in association with presence of varices is planned in the future

P-0807

Histoacryl® therapy of bleeding gastric varices; the experience of tertiary care hospitals

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Introduction: Gastric variceal bleeding is not only life threatening, also contributes to high rates of morbidity, recurrent hospitalizations. **Aim:** To evaluate the efficacy and safety of endoscopic injection of N-butyl-2-cyanoacrylate (NBCA) for treatment of bleeding gastric varices (GV).

Methods: Analysis of prospectively collected data of a cohort of patients with GV who underwent endoscopy for the treatment of bleeding GV from April 2013 to September 2015. Patients with gastric variceal bleeding underwent endoscopic treatment with a mixture of NBCA and Lipiodol. The success of GV eradication was assessed by repeat endoscopy after 3 weeks of intervention. Successful hemostasis, rebleeding rate and complications were observed.

Results: The cohort consisted of 33 consecutive patients that had undergone NBCA injection for GV. The mean age was 51 ± 10 years. The mean follow up was 16 ± 8 months and the most common cause for GV was hepatitis C related liver cirrhosis (51.5 %). Child-Pugh score at presentation for was A-21 %; B-79 %, and median MELD score at admission was 10. A median mixture volume of 4.5 mL, in 1 to 2 injections, was used, with immediate hemostasis rate of 100 % and early rebleeding rate 3.8 %. Mortality rate was 3.8 %. No immediate or longterm complications of NBCA injection occurred in any of these cases during the time of follow up. **Conclusion:** NBCA injection of GV is a safe and successful therapeutic intervention. Patients with very early rebleeding was at higher risk of death. A minimum of 2 endoscopic sessions is required to significantly decrease the risk of rebleeding.

P-0808

Outcome of patients treated with N-butyl-2-cyanoacrylate in gastric variceal bleeding**Muhammad Mansoor UL Haq, Abdul Latif Memon, Mansoor Asad Kalwar, Kailash Raj Makhejani**

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Background: Gastric variceal bleeding (GVB) is generally more severe than bleeding from esophageal varices (EVs), but it occurs less frequently. N-butyl-2-cyanoacrylate is being successfully used for the treatment of gastric fundal variceal bleeding. In Canadian study Hemostasis after FVO was achieved in 35 patients (95 %) With active bleeding & all-cause mortality was 28.6 % (10 of 35).

Objective: Aim of our study was to determine outcomes of Patients treated with N-butyl-2-cyanoacrylate.

Methods: A Retrospective study was conducted at Liaquat National Hospital, Karachi between March 2012 to June 2014 by viewing medical records and endoscopy reports. Total 31 patients were enrolled with gastric variceal bleed that underwent endoscopic injection of N-butyl-2-cyanoacrylate; we examined the mortality rate, hemostasis rate, hospital stay, and complication of procedure.

Results: A total of 31 patients, out of which 18 (58.1 %) were male; the mean age was 55.23 ± 8.778 years. Of these patients 23 (74.2 %), had concomitant esophageal varices, Child-Pugh class-A, B, C were seen in 4, 20 and 7 patients (12.9, 64.5 % and 22.6) respectively. Average duration of hospital stay was 5 to 8 days in 22 cases (71.0 %). Hemostasis was achieved in 27 patients (87 %). Overall mortality rate was 3 out of 31 (9.7 %). one patient was referred for TIPSS. No complications from cyanoacrylate injection were observed.

Conclusion: In our study hemostasis was achieved in majority of cases, therefore hospital stay and mortality due to bleeding was reduced after achieving hemostasis. Standardized injection technique and regimen ensures the success and safety of N-butyl-2-cyanoacrylate injection for the treatment of gastric fundal varices in experienced hands.

P-0809

Gastric varices in cirrhosis vs EHPVO and response to endoscopic n-butyl-2-cyanoacrylate injection**Shivakumar Varakanahalli¹, Barjesh C. Sharma¹, Siddharth Srivastava¹, Jatinder P. Singh¹, Piyush K Gupta²**¹G B Pant Institute of P G Medical Education and Research, New Delhi, India; ²Maulana Azad Medical College, New Delhi, India

Aims and objectives: To compare the prevalence and types of gastric varices in cirrhosis versus extrahepatic portal venous obstruction (EHPVO) and the results of endoscopic N-butyl-2 cyanoacrylate (NBC, glue) injection.

Methods: 454 patients presenting with bleeding from gastric varices between August 2010 and August 2015 were retrospectively analyzed.

Results: Of 454 patients, 64 % (n = 292) were cirrhotics and 36 % (n = 162) had EHPVO. Distribution of types of gastric varices showed GOV1 in 16.4 % (n = 48) of cirrhotics vs. 7.4 % (n = 12) of EHPVO, GOV2 in 77 % (n = 224) of cirrhotics vs. 53 % (n = 86) of EHPVO, IGV1 in 39 % (n = 64) of patients with EHPVO vs. 7 % (n = 20) cirrhotics. The patients were treated with NBC injections. The mean volume of glue injected was 2.89 ± 1.59 ml over a median of 1 session (range 1 to 5). The total volume of glue required was

lower in cirrhotics (2.44 ± 1.17 ml vs. 3.7 ± 1.9 ml, $p < 0.01$). 117 (40 %) of cirrhotics required >1 sessions of glue injection as compared to 102 (63 %) of EHPVO patients. Over mean follow up of 14.7 months, rebleeding (10 vs. 13 %) and mortality (15 vs. 2.5 %) was higher in patients with cirrhosis and EHPVO.

Conclusions: In patients with bleeding from gastric varices, GOV2 is more common in cirrhotics and IGV1 in patients with EHPVO. Patients with EHPVO required higher total volume of glue and more glue sessions for gastric varix obturation.

P-0810

Long-term outcomes of B-RTO for prevention of first or second bleeding of gastric fundal varices**Hisashi Hidaka, Tomoyoshi Inoue, Kousuke Kubota, Keiko Yamane, Juichi Takada, Yoshiaki Tanaka, Yusuke Okuwaki, Takahide Nakazawa, Akitaka Shibuya, Wasaburo Koizumi**

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Objective: Balloon-occluded retrograde transvenous obliteration (BRTO) is a highly effective treatment for gastric fundal variceal (FV) bleeding, but the long-term efficacy is not well known. Therefore, we investigated the long-term effects of BRTO on the prevention of bleeding and mortality.

Methods: BRTO was performed in 154 cirrhotic patients with FV from March 1993 through May 2015. Eight patients had been experiencing bleeding. Forty-one patients had recent bleeding treated by endoscopic CA injection or balloon tamponade. One hundred five patients had varices that would likely bleed (rapid increase) but without bleeding. There were 90 men and 64 women in the study with an age range of 27–86 years (median age 64).

Results: Eleven patients had bleeding from IGV1, esophageal varices (EV) or ectopic varices after BRTO. Twenty-eight patients had EV progress within 1 year after BRTO. Cumulative non-bleeding rate after BRTO was 96.9 %, 91.9 % and 90.3 % for 1, 3 and 5 years, and the cumulative survival rate was 90.5, 73.7 and 60.4 % for 1, 3 and 5 years (observation period: 3–7987 days, median: 2661 days). There were no severe complications or mortality caused by BRTO. Hepatic cancer or liver failure irrelevant to this treatment was the main cause of death. The following factors were significant independent predictors of survival (non-complications of hepatic cancer: $p < 0.001$, HR: 2.837; 95 % CI: 1.528–5.266; non-complications of EV: $p < 0.001$, HR: 2.700; 95 % CI: 1.567–4.651).

Conclusions: The long-term outcomes of BRTO for FV were excellent. BRTO should be a standard treatment for these patients.

P-0811

Endoscopic variceal obliteration vs BRTO as a prophylactic treatment for gastric varices**Hyung Joon Yim¹, Jung Wan Choe¹, Seung Hwa Lee², Hwan Hoon Chung², Sang Jun Suh¹, Seung Young Kim¹, Jong Jin Hyun¹, Sung Woo Jung¹, Young Kul Jung¹, Ja Seol Koo¹, Ji Hoon Kim³, Yeon Seok Seo⁴, Jung Eun Yeon³, Sang Woo Lee¹, Kwan Soo Byun³, Soon Ho Um⁴**¹Department of Internal Medicine, Korea University Ansan Hospital, Ansan, Korea; ²Department of Radiology, Korea University College of Medicine, Seoul, Korea; ³Department of Internal Medicine, Korea

University Guro Hospital, Seoul, Korea; ⁴Department of Internal Medicine, Korea University Anam Hospital, Seoul, Korea

Background: No single effective method has yet been established for the prophylactic treatment of gastric varices.

Methods/aims: We retrospectively analyzed patients with gastric varices, who had undergone either endoscopic variceal obliteration (EVO) or balloon-occluded retrograde transvenous obliteration (BRTO) as a prophylactic treatment.

Results: Total 84 patients were consisted of 55 patients in EVO group and 29 patients in BRTO group. No difference was observed in the clinical profiles of patients, including age, gender, Child-Pugh score, etiology of liver cirrhosis, and presence of hepatocellular carcinoma, between the EVO and BRTO groups. There was also no difference with respect to endoscopic features of gastric varices including F-component and location. As primary end points, the gastric varices were disappeared partially or completely in 50 patients in EVO group, and 27 patients in BRTO group. (90.9 vs 93.1 %, $p = 0.542$). At the complete eradication rate, there was also no difference between two groups. (49.1 % vs 65.5 %, $p = -0.150$) However, 12 patients in EVO group bled from gastric varices after treatment during the median follow-up of 28 months, compared to only one case in BRTO group. (21.8 vs 3.4 %, $p = 0.027$) In addition, there were no differences in worsening in the endoscopic classification of esophageal varices or amounts of ascites. All-cause mortalities were similar in both.

Conclusions: EVO and BRTO are equally effective for eradication of gastric varices with similar frequencies of complications and mortalities. However, BRTO proved more effective in preventing bleeding from gastric varices.

P-0812

Gastric varices successfully treated by shunt occlusion-endoscopic injection therapy: A case report

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A 64-year-old man with hepatitis C cirrhosis was admitted for the treatment of isolated gastric varices. He had a past history of hepatocellular carcinoma (HCC) which was treated by partial hepatic resection. His hepatic function was class A, score 5 according to the Child-Pugh classification. Computed tomography (CT) showed that the drainage route included a splenorenal shunt and the inferior phrenic vein. Esophagogastroduodenoscopy (EGD) revealed that they were white (Cw), nodular form (F2) and located in the cardia and fundus (Lg-cf). Balloon-occluded retrograde transvenography (BRTV) showed that they were not opacified because the inferior phrenic and pericardiacophrenic veins were working as a collateral vein. To perform balloon-occluded retrograde transvenous obliteration (BRTO), we intended to advance a microcatheter into the collateral vein via the left renal shunt and inferior phrenic vein. However, it was indented and we could not occlude them. Thus, we intended to treat the patient using shunt occlusion-endoscopic injection therapy (SO-EIS). We occluded the outlet of the left renal shunt with a balloon catheter, directly punctured the gastric varices via EGD, and injected 5 % ethanolamine oleate (EOI) until the gastric varices and flow vein were opacified, and then injected cyanoacrylate. One week later, CT showed complete thrombosis of the varices and

flow vein. BRTO is difficult to perform in cases where we cannot occlude the collateral vein. A direct puncture via EGD has a high risk for flowing EOI into the systemic circulation. SO-EIS may be a promising alternative treatment.

P-0813

Comparison of obliteration of gastric varices and technical success between BRTO vs PARTO

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Introduction: Balloon-occluded retrograde transvenous obliteration (BRTO) has widely been accepted for treating GV and preventing GV bleeding. Recently, vascular plug and gelatin sponge-assisted RTO (PARTO) is used in place of BRTO to overcome adverse events caused by ethanolamine infusion or balloon rupture. This study compares the obliteration of targeted GV and technical success of PARTO with BRTO.

Methods: Total 59 patients with gastric varices grade 2 or more who had undergone PARTO ($n = 30$) or BRTO ($n = 29$) from Jan 2006 to Dec 2014 were retrospectively evaluated. Vascular plug (Amplatzer Vascular Plug; AGA Medical, Golden Valley, Minn) and absorbable gelatin sponges (Gelfoam; Upjohn, Kalamazoo, Mich) were used for PARTO. Gastrointestinal endoscopy and/or computed tomography (CT) were performed after procedures for ascertaining the obliteration of GV.

Results: The median age of patients was 56 (IQR; 62–47) and male predominant (48/59, 81.4 %). Two cases in each group were excluded due to follow-up loss. The technical success rate was significantly higher in PARTO group (29/30, 96.7 %) compared to BRTO group (18/29, 62.1 %) ($p = 0.001$). Among these patients, the GV eradication rate of PARTO group (26/28, 92.9 %) was also significantly higher than BRTO group (11/17, 64.7 %) ($p = 0.039$). In multivariate logistic regression analysis for predicting GV eradication, procedure (PARTO vs. BRTO) was an independent risk factor after adjusted by age and MELD score (odds ratio 5.98, $p = 0.043$). 3 cases of GV bleeding were developed only in BRTO group.

Conclusions: PARTO seems to be a more technically successful and clinically effective procedure for the treatment of GV compared to BRTO.

P-0814

The efficacy of danaparoid sodium on portal thrombosis in patients with hepatic cirrhosis

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Background: Portal thrombosis is a life-threatening complication in patients with hepatic cirrhosis. Medical treatments for thrombosis such as administration of heparin and urokinase have not always succeeded. Danaparoid sodium is known to have about 23-fold

activity against Xa comparing to the activity of heparin sodium, and is reported that frequency of bleeding is a one-tenth of that by heparin. We tried to administer danaparoid to investigate the efficacy and safety.

Methods: We conducted thrombolytic treatments for portal thrombosis in 15 patients with hepatic cirrhosis who admitted Yamagata University Hospital from June, 2007 to September, 2015. These patients were divided into two groups: danaparoid group and heparin group. Eight patients in the danaparoid group were intravenously administered 1250 units of danaparoid twice a day for 14 days. The remaining seven in the heparin group received continuous infusion of heparin sodium (10,000 IU/day) and urokinase (60,000–120,000 IU/day) for 7–12 days.

Results: In the danaparoid group, retardation of portal thrombosis assessed with CT scan was observed without any complications including bleeding in all of the seven patients in the danaparoid group. Three of the seven were supplemented AT-III for 3 days. However, the retardation was seen only in five of the seven patients in the heparin group. In addition, the two patients without the retardation died of liver failure.

Conclusion: Danaparoid sodium could be administered easier and without complication and retard portal thrombosis in all patients. The result from this study warrants clinical trials including RCT to test the efficacy of the agent on portal thrombosis in hepatic cirrhosis.

P-0815

Danaparoid sodium thrombolytic therapy followed by warfarin in cirrhotic portal vein thrombosis

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Background: Portal vein thrombosis (PVT) is a complication of cirrhosis that reduces the hepatic reserve and causes variceal bleeding. The therapeutic efficacy of danaparoid sodium (DS), a heparinoid anti-coagulation factor Xa, for PVT has been reported.

Methods: We retrospectively analyzed 41 hospitalized cirrhotic patients: 16 hepatitis C virus, 5 hepatitis B virus, 20 others; the model for end-stage liver disease (MELD) score 8.6 ± 4.7 ; platelets $80 \pm 40 \times 10^3/\mu\text{L}$; 3 esophageal varices F0, 16 F1, 5 F2, 0 F3, and 17 unknown. DS 2500 units were administered daily ($n = 41$, mean duration: 9.5 days), followed by oral warfarin (prothrombin time-international normalized ratio: 1.5 ± 0.3) in outpatient clinic ($n = 16$, 25.8 weeks). The volume of PVT (PVTV) measured with a three-dimensional-image analyzer ($n = 28$), serum D-dimer ($n = 29$), and scintigraphic portal shunt indices (normal, $<10\%$; $n = 6$) were monitored.

Results: Thrombi formed at one site in 25 patients (18 portal, 4 superior mesenteric, and 3 splenic veins) and at two or more sites in 16. At the end of DS therapy, the PVTV decreased to $55.1 \pm 40.2\%$ of baseline ($8.6 \pm 10.3 \text{ cm}^3$, $p < 0.0001$), D-dimer decreased from $11.8 \pm 12.6 \mu\text{g/mL}$ to $7.0 \pm 7.4 \mu\text{g/mL}$ ($p = 0.007$), and the shunt indices decreased from $62.4 \pm 10.5\%$ to $56.9 \pm 7.1\%$ ($p = 0.250$). During DS therapy, Grade 2 intraperitoneal bleeding occurred in one patient (2.4%). During follow-up, PVTV increased in 33.3% of the

patients, MELD score increased in 37.5%, platelets decreased in 50.0%, and varices grade increased in 18.2%. Conclusions: PVT could be resolved with DS with safety. Warfarin did not always maintain the effects of DS.

P-0816

Treatment of portal vein thrombosis in cirrhosis: a multicenter real life study

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Aims: Portal vein thrombosis (PVT) is common in cirrhosis and can be cause or consequence of disease progression. Small studies have shown benefit of anticoagulation. We assessed anticoagulation on this population for safety, efficacy and survival. Method: Cirrhotics with PVT, the majority decompensated, were included in a data base retrospectively (before 2013) and prospectively (2013-10/2014). Demographics: 76 patients (61 male), median age 67 (36–88) and BMI 26.8 (17.9-32.4), etiologies: alcoholic 40%, HBV 25% and HCV 16%, HCC in 47.4%. Median MELD-score was 12 (6-25), Child-Pugh 7 (5-12). 79.5% of patients were decompensated at PVT diagnosis, 89.6% had varices (62.5% large), 33% high-risk signs. Main trunk involvement in 77%, cavernoma existed in 17%. 51 patients anticoagulated (65% LMW-Heparin, 25% warfarin). Pre-treatment varices eradicated in 30%, while a 75% of patients were on beta-blockers. Survival was inferior for treated (median 15 months) albeit not statistically (ns)-significant ($p = 0.311$); HCC patients had n.s. trend for inferior survival as for alcoholics ($p = 0.06$). PV patency 28.5% of treated (n.s). Portal hypertension (PHT) bleeding identified in 24 patients (31.6%), only in 6 after PVT diagnosis, 2 under treatment (fatal). Two patients experienced non-PHT gastrointestinal bleeding (1 fatal). Majority (75%) of deaths were due to liver failure and HCC-related causes.

Conclusion: Treatment of PVT in cirrhotics is feasible with acceptable side-effects. Alcoholic etiology and HCC have negative impact on survival. In our cohort there was no clear benefit of treating PVT in cirrhotics, mainly decompensated. A bias, commencing anticoagulation in patients with more advanced disease, cannot be excluded.

P-0817

Liver stiffness measurement <12 kPa excludes large varices in patients with early cirrhosis

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Background/aims: Screening gastroscopy for varices is recommended when cirrhosis is diagnosed. However, the benefit of

screening gastroscopy in patients with early cirrhosis remains unclear. We hypothesize that liver stiffness measurement (LSM) can risk-stratify patients who would most benefit from screening gastroscopy and thus avoid unnecessary gastroscopy.

Methods: We performed a retrospective study of patients with early cirrhosis (defined as the presence of coarse echogenicity on imaging but without clinical or biochemical evidence of cirrhosis) who had LSM followed by screening gastroscopy. The ability of LSM to predict presence of large (Grade >2) varices was assessed using AUROC statistics.

Results: 130 patients (mean age 61 ± 12 years) with early cirrhosis underwent baseline LSM. Etiology was hepatitis B (55 %), NAFLD (15 %), cryptogenic (13 %), hepatitis C (5 %). All had compensated Child-Pugh A cirrhosis with mean albumin, bilirubin, prothrombin time and platelet counts of 40 ± 4 g/L, 16.7 ± 7.6 mmol/L, 10.9 ± 0.8 s and $177 \pm 21 \times 10^9/L$, respectively. 84 (65 %) subsequently underwent screening gastroscopy. Large varices were present in 18/84 (21 %). LSM was a good predictor for presence of large varices (AUROC 0.814, 95 % CI 0.705–0.923, $p < 0.001$). Optimal LSM to provide >90 % sensitivity for detection of large varices was 12 kPa (sensitivity 94.4 %, specificity 43.9 %, PPV 31.5 %, NPV 96.7 %). Large varices were found in only 3.3 % of patients with LSM <12 kPa compared to 31.5 % in LSM >12 kPa ($p = 0.002$).

Conclusion: In patients with early cirrhosis, LSM <12 kPa has a high negative predictive value for excluding large varices. Such patients may not require screening gastroscopy immediately and can be monitored.

P-0818

LSPS identifies esophageal varices in chronic liver disease

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Background and aim: Noninvasive methods are needed to identify esophageal varices (EV) in patients with chronic liver disease (CLD). To this end, we evaluated liver stiffness (LS)-spleen diameter-to-platelet ratio risk score (LSPS) in predicting EV and high risk of EV among Japanese CLD patients.

Methods: A total of 360 patients with CLD who had undergone endoscopy, LS measurement, and ultrasonography between 2012 and 2014 were enrolled. Clinical data were compared with those for other noninvasive markers (platelet count, aspartate aminotransferase-to-platelet ratio, FIB-4 index, and platelet-to-spleen ratio), spleen size, LS, and controlled attenuation parameter. Diagnostic applicability was assessed by the area under the receiver operating characteristic curve (AUC) and predictive values along with multivariate logistic regression.

Results: LSPS was significantly correlated to the grade of EV ($\rho = 0.478$, $P < 0.001$) and was superior to the other noninvasive indices for determination of EV and high EV risk. LSPS was independently associated with EV and high EV risk by multivariate logistic regression analysis. The cutoff values of LSPS for EV and high EV risk were 1.0 and 4.4, respectively, at which the AUC, negative predictive value, and accuracy were 0.847 (95 % confidence

interval: 0.784–0.910), 92.2 %, and 83.1 % and 0.854 (95 % confidence interval: 0.729–0.978), 92.5 %, and 86.2 %, respectively.

Conclusions: LSPS may also identify EV and a high risk of EV in CLD in Japan. The clinical values of LSPS for EV and high EV risk merit further validation in larger prospective studies.

P-0819

Effect of time interval for prevention of variceal rebleeding in patients without active bleeding

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Background: The optimal timing of endoscopic intervention has not been determined in cirrhotic patients found on initial endoscopy to have blood clots in the stomach, without active bleeding varices or stigmata. Aim This study compared outcomes in patients who underwent emergency endoscopic variceal ligation (EVL) and elective interventions.

Methods: This study included 28 cirrhotic patients who underwent emergency prophylactic EVL and 41 who underwent elective endoscopic intervention between January 2009 and June 2014 after initial endoscopy showed blood clots in the stomach without active bleeding including stigmata. Clinical outcomes, including rebleeding, 6-week mortality, and 6-week rebleeding-free survival rates, were analyzed.

Results: The rebleeding rate was significantly higher in the emergency than in the elective group (8/28 [28.6 %] and 3/41 [7.3 %], $P = 0.041$). Multivariate analysis showed that emergency prophylactic EVL (odds ratio [OR], 7.4; 95 % confidence interval [CI], 1.6 to 34.8) and advanced liver cirrhosis (Child-Pugh score C; OR 10.6; 95 % CI, 1.4 to 80.8) were significantly associated with an increased risk of rebleeding. Subgroup analysis showed that the presence of gastric varices was associated with the rebleeding in the emergency group (OR 12.0, 95 % CI, 1.7–83.5). Six-week rebleeding-free survival rates were similar in emergency and elective patients without gastric varices (83.3 vs. 80.8 %, $P = 0.733$). **Conclusions** Elective intervention may be a safer strategy for preventing gastric variceal rebleeding in cirrhotic patients with blood clots in the stomach, but without active bleeding or stigmata. Emergency EVL may be effective in patients without gastric varices.

P-0820

Long-term outcome of cirrhotic patients with bleeding esophageal and gastric varices

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Aim: Although endoscopic and B-RTO procedures improved a success rate of initial hemostasis of bleeding esophageal and gastric varices (EV/GV), prognosis of a patient remains unclear since

bleeding from the lesions may recur. Long-term outcome of patients showing EV/GV bleeding was evaluated.

Methods: The subjects were 251 patients with hemorrhagic EV/GV seen between January 2006 and July 2015. Factors associating survival and rebleeding were evaluated.

Results: Bleeding from EV or gastric cardiac varices (GCV) was seen in 212 patients, while from gastric fundal varices (GFV) in 39 patients. Child-Pugh classes were A, B and C in 56, 124 and 68 patients, respectively. EVL were performed in 203 patients, EIS with ethanalamine oleate in 10 patients and EIS with cyanoacrylate in 34 patients. B-RTO was done in 22 patients with GFV following EIS with cyanoacrylate. The overall hemostasis rate was 96 %. The cumulative rebleeding rates at 1, 3 and 5 years were 28, 35 and 35 %, respectively, in patients with bleeding from EV or GCV, while rebleeding did not occur in patients showing GFV bleeding. The survival rates at 1, 3 and 5 years were 73, 62 and 56 %, respectively; 70, 59 and 54 % in cases of EV or GCV and 88, 80 and 62 % in cases of GFV. Multivariable analysis identified HCV infection, Child-Pugh class-C and HCC as significant factors associating survival, and age and HCC associating rebleeding.

Conclusions: Therapeutic strategy combined with endoscopic and B-RTO procedures was useful to achieve a favorable long-term outcome in patients showing EV/GV bleeding.

P-0821

What predicts short term mortality in patient with variceal bleeding?

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Background: Acute variceal bleeding may be fatal in patients with liver cirrhosis. They may miss an opportunity of endoscopic exam due to poor medical condition. If there were experienced endoscopist, early endoscopic therapy is preferable. The blood urea nitrogen to creatinine (BUN/Cr) ratio is a marker of dehydration and an index of gastrointestinal bleeding severity. We investigated if BUN/Cr ratio reflects current variceal bleeding and predicts 7-day mortality.

Methods: From January 2012 to November 2014, the patients who admitted to emergency room for hematemesis or melena were enrolled. They were diagnosed as liver cirrhosis and suspected as variceal bleeding. The current variceal bleeding was defined in the cases of oozing and/or spurting or stigma with blood pool were noted by endoscopic exam.

Results: Total 371 patients were enrolled. Mean age was 60 years, male proportion was 77 %. Laboratory findings was followed as: Hb 9.1g/dL, platelet 113 k/microL, PT INR 3.17, albumin 3.2 g/dL, total bilirubin 2.6 mg/dL, BUN 30 mg/dL, Cr 1.17 mg/dL, Osmolality 308 mOsm/kg. Liver status was followed as: Child-Pugh class A/B/C 32/46/22 %, HCC 23 %. Vital signs as: heart rates 98 bpm, systolic/diastolic blood pressure 116/67 mmHg. Multivariate analysis revealed that age and BUN/Cr ratio reflect current variceal bleeding. (Age: Odds ratio 0.976, C.I. 0.957–0.996, P-value 0.018; BUN/Cr ratio: odds ratio 1.028, C.I. 1.014–1.043, P-value 0.000) Multivariate analysis revealed that PT prolongation predicts 7-day mortality. (PT INR: Odds ratio 6.569, C.I. 2.461–17.531, P-value 0.000).

Conclusion: The BUN/Cr ratio reflects current variceal bleeding. The PT prolongation predicts 7-day mortality.

P-0822

Clinical implication of portal hypertensive colopathy accompanied by cirrhotic patients

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Background: Portal hypertensive colopathy (PHC) is presented as mucosal/submucosal abnormalities of colon not rarely observed in cirrhotic patients. However, no consensus is established in aspects of the definition of colonic lesions and its clinical relevance. This study was performed to identify the pattern/frequency of colonic changes and to evaluate their clinical implication in cirrhotic patients.

Methods: One hundred-thirty nine patients with liver cirrhosis who underwent diagnostic colonoscopy, after excluding the patients with a previous history of gastrointestinal intervention, were enrolled in the present study. Medical records along with endoscopic findings of enrolled patients were compared with those of age- and sex-matched health examinees.

Results: PHC indicated as mucosal/submucosal abnormalities of colon (hemorrhoids/anorectal varices, angiodysplasia, erythema/hyperemia, edema, granularity, and friability/spontaneous bleeding) were more frequent in cirrhotic patients than controls (38.8 vs. 3.6 %, <0.05). The most frequent colonic abnormalities were hemorrhoids/anorectal varices (20.9 %), the next was angiodysplasia (10.1 %). Cirrhotic patients with colonic abnormalities were more likely to have higher grade esophageal varices than cirrhotics without colonic abnormalities (44.4 vs. 25.9 %, <0.05). However, no difference was noted in age, sex, etiology of cirrhosis, Child-Pugh classification, MELD score between the patients with or without colonic abnormalities. One-, three-, and five-year gastrointestinal bleeding rates of cirrhotic patients with colonic abnormalities were higher than those of patients without colonic abnormalities (3.7, 7.4, and 11.1 vs. 0, 1.2, and 1.2 %, respectively, <0.05).

Conclusions: PHC including hemorrhoids/anorectal varices, angiodysplasia, non-specific inflammation, and mucosal friability were frequent in cirrhotic patients, implicating to be a risk factor of gastrointestinal bleeding.

P-0823

Non-selective beta-blockers and physical function

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Background: β -blockers (BB) are the main pharmacologic therapy for prophylaxis against variceal bleeds. While fatigue and weakness are commonly reported with BB use in the general population, little is known of the impact of these side effects on physical activity and function in patients with cirrhosis.

Methods: Cirrhotics at a single center underwent testing of physical function using the Short Physical Performance Battery (SPPB: gait + balance + chair stands; <9 = impaired), exhaustion using the Fried Frailty questionnaire, and physical activity using the Minnesota leisure-time activity survey. BB use was assessed from medical chart review. Multivariate analyses were performed using logistic and linear regression.

Results: Of 344 cirrhotics: 35 % female, median age 60, MELD 15, 53 % were on BB. Compared to those not on BB, patients on BB were similar except for %female (25 vs. 46 %). In patients taking vs. not taking BB, rates of exhaustion (51 vs. 52 %) functional impairment (18 vs. 14 %), and physical activity levels (129 vs. 218 kcal/week) were similar. In adjusted analyses, BB were still not associated with exhaustion (OR 0.97, 95 % CI 0.6 to 1.5). After adjusting for heart rate and Child Pugh score, BB usage was associated with functional impairment (OR 1.92, 95 % CI 1.00 to 3.81) and a trend toward low physical activity (coeff. -186 , 95 % CI -399 to 26.9).

Conclusion: In patients with cirrhosis, BB use is associated with low physical activity and functional impairment, even after adjustment for Child Pugh score. Consideration of these risks should be given when prescribing BB to the most frail and sedentary cirrhotics.

P-0824

Heart failure and liver cirrhosis in patients with ascites

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Background: Among the liver cirrhosis patients with ascites, especially in elderly, there were many complicated cases with heart disease. Generally, for the ascites due to the heart failure, total protein concentration in ascites was assumed more elevated than the liver cirrhosis. However, a significant number of cases were still misclassified. We examined serum and ascitic markers to investigate the contribution of heart failure and liver cirrhosis in patients with ascites. **Methods:** From January 2010 to June 2015, patients with ascites that were admitted to the Saitama Medical Center, Jichi Medical University, were assessed for eligibility. Patients underwent physical examination, rest echocardiography, and collection of blood and ascitic fluid samples for analysis. 20 patients were included in the study. **Results:** Both serum BNP ($r = 0.84$) and LDH/ALT ratio ($r = 0.80$) showed a high correlation coefficient with total protein concentration in ascites. The FIB-4 index, a predictive index of liver fibrosis, was higher in the liver cirrhosis patients. The FIB-4 index showed intermediate negative correlation with total protein concentration in ascites ($r = 0.52$). Seven patients needed treatment of collaboration with cardiovascular specialist, showed significantly higher serum BNP level (average 1650 vs 101 pg/ml) compared with non-collaboration cases. **Conclusions:** In addition to BNP and total protein concentration in ascites, LDH/ALT ratio was also suggested to be useful for differential diagnosis with heart failure-related ascites. There were many complicated cases with heart disease and liver cirrhosis, which needed the treatment for each pathophysiology. It is important to evaluate comprehensive assessment of general status using multiple markers.

P-0825

Natural history of grade 1 ascites in patients with liver cirrhosis

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Aim: The natural history of cirrhotics and grade 1 ascites is unclear. Aim of the study was to address this issue and identify predictive factors of survival.

Method: 100 patients with grade-1 ascites were analyzed retrospectively (mean age: 59.9, males: 88 (88 %), etiology: alcohol/HBV/HCV/autoimmune/other: 57/19/13/2/9, CPS (median): 8 (5–13), CP-class A/B/C: 18/60/22, MELD (median): 12.5 (6–25), hepatocellular carcinoma: 11. None was under treatment with diuretics. Control group were 146 patients with Grade 2/3 ascites (including grade-1 under diuretics) and 174 without ascites. Cox regression was used for survival analysis. Diuretics were initiated in 58 patients at baseline. At the latest follow-up, 29 had no ascites, 33 had grade-1, 17 had grade-2 and 21 had grade-3 ascites. Initiation of diuretics was not correlated with ascites regression ($p = 0.292$) or not worsening of grade ($p = 0.785$). Liver-specific treatment commenced in 58 patients but was not correlated with ascites regression ($p = 0.33$). Seven developed spontaneous bacterial peritonitis (SBP), on diuretics; that time 1 patient had grade-1; the remaining 6 patients grade 2/3. Mortality (36 %) was lower, compared to patients with grade 2/3 ascites ($n = 73$, 50 %; $p < 0.001$), but similar to non-ascitic patients ($n = 57$, 32.8 %; $p = 0.087$). In the multivariate, overall survival in Grade-1 ascites associated significantly with: low albumin (HR: 0.43, $p = 0.017$), high creatinine (HR: 1.383, $p = 0.006$), advanced age (HR: 1.047, $p = 0.018$).

Conclusion: 40 % of cirrhotics and grade-1 ascites developed worsening ascites despite initiation of diuretics and/or liver-specific treatment. Mortality risk was similar to non-ascitic patients. SBP risk was low and occurred in more advanced stages.

P-0826

N-terminal pro B-type natriuretic peptide is useful diagnostic markers for the new onset ascites.

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Background: Serum ascites albumin gradient (SAAG) is very useful marker to identify the cause of ascites. But it is sometimes difficult to distinguish ascites from liver cirrhosis (LC) and heart failure (HF) for both conditions are SAAG is greater than 1.1 g/dL. The aim of present study is to evaluate N-terminal pro B-type natriuretic peptide (NT-proBNP) as one of diagnostic tool identifying the uncertain origin ascites.

Methods: From October 2008 to February 2014, A total 341 patients were diagnosed the new onset ascites in Myongji Hospital. We retrospectively reviewed the medical records of these patients and analyzed 61 patients who were examined both hepatologic study (ascites lab, liver function test and image) and cardiologic study (NT-proBNP, echocardiography). These patients were divided into three groups: LC group (N = 34), HF group (N = 10) and peritoneal disease (PD) group (N = 17).

Results: The median values of NT-proBNP were 376.4 of LC, 3819.0 in HF and 622.4 in PD group. It showed statistically significant difference between LC and HF group ($p = 0.001$), but no significant

difference between another two group comparison. We suggested the diagnostic cutoff value distinguished ascites from LC and HF 927.5 pg/ml (Sensitivity 87.5 %, Specificity 67.3 %). It is more valuable than SAAG.

Conclusions: Serum NT-proBNP is one of useful biomarkers for identifying the cause of ascites, especially from the HF. It may be very useful process for the work up of patients with new onset ascites including NT-proBNP and diagnostic paracentesis, we could immediate find the accurate cause of ascites and helpful management.

P-0827

Renal vein dilation predicts the prognosis of cirrhotic ascites

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Background and aims: Renal venous hypertension is known to be associated with worsening of renal function in patients with decompensated heart failure. Intra-abdominal hypertension including cirrhotic ascites also leads to renal venous hypertension. We aimed to clarify the effect of renal vein dilation on cirrhotic ascites.

Methods: The left renal vein diameter was measured in 100 patients with cirrhotic ascites using computed tomography.

Results: The median overall survival (OS) for patients with a large left renal vein was less than that for patients with small renal vein diameter ($P < 0.001$; 1.9 vs. 32.0 months). Multivariate analysis revealed larger renal vein diameter (hazard ratio [HR]: 3.842; $P < 0.001$; 95 % confidence interval [CI]: 1.943–7.599) and a high model for end-stage liver disease–Na score (HR: 4.055; $P < 0.001$; 95 % CI: 2.123–7.747) were significant independent predictors of poor prognosis. Left renal vein diameter was correlated with direct bilirubin levels, PT-INR, NH₃, and age. Multivariate analysis of left renal vein diameter >11 mm revealed higher serum direct bilirubin levels (Odds ratio: 2.450; $P = 0.034$; 95 % CI: 1.070–5.610) as an independent predictor of left renal vein diameter >11 mm.

Conclusions: Renal vein dilation is an independent predictor of survival in patients with refractory cirrhotic ascites, and renal vein diameter was correlated with serum direct bilirubin levels

P-0828

Ascitic fluid cholesterol may be a good parameter to assess the severity of liver cirrhosis

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Asses the severity of liver cirrhosis remains a clinical problem. Appropriate management of liver cirrhosis depends on proper staging. To asses the severity, various staging system were used. The present study was conducted at the Department of Hepatology, Bangabandhu Sheikh Mujib Medical University (BSMMU) to see the 'value of ascitic fluid cholesterol to asses the severity of liver cirrhosis'. A total 50 patients with cirrhotic ascites were enrolled who met the inclusion criteria. Most of the cirrhotic patients are in Child-Pugh (CP) stage C (72 %, $n = 36$), 28 % patients found in Child-Pugh stage B ($n = 14$), none of the patient with cirrhotic ascites found in Child-Pugh stage A. Ascitic fluid cholesterol in cirrhotic patients with CP stage B is 10.44 ± 6.38 mg/dl, whereas in CP stage C it is 7.83 ± 5.86 mg/dl. But the result is not statistically significant ($p > 0.05$). Although the result is not statistically significant, but it is clear that ascitic fluid cholesterol gradually decreases as the worsening of the liver function. Here sample size is small which may be the cause of statistically non-significant result and large scale study may be needed. In view of simplicity, easy availability and cost effectiveness, ascitic fluid cholesterol may be a good parameter for staging liver cirrhosis.

	Test I-V	Test I-IV	Test I-III	Test I-II
Patient No 30	5=7	5=6.4	5=5.8	5=2.1
Patient No 58	5=4.5	5=2.7	5=3.2	5=3.5
Patient No 32	3.1=3.3	3.1=3.2	3.1=3.6	-
Patient No 28	2.8=2.5	2.8=2.5	2.8=3	-
Patient No 31	2.7=2.1	2.7=2	2.7=2	-
Patient No 33	2.5=2	2.5=2	2.5=2	-
Patient No 35	2.5=2	2.5=2	2.5=2.4	-
Patient No 22	2=2.3	-	2=2.1	2=2.8
Patient No 36	3=2	-	3=2.3	-
Patient No 60	-	-	2.3=2.1	-
Patient No 27	-	-	2.2=2	-
Total	9 (30%)	7(23%)	11(36%)	3(10%)

	P-14r	N-20r	P-14l	N-20l	P14r-N20r	P14l-N20l
Patient	13.8=1.4	19.3=1.5	14=1.6	19.2=2.2	4.5=0.7	5.1=0.7
Control group	12.9=1.3	17.1=1.1	12.9=1.3	16.9=1.2	4.1=0.9	3.8=1.1
P value	P<0.00005	P<0.001	P<0.0002	P<0.016	P<0.001	P<0.001

P-0829

Denver peritoneovenous shunt selectively enriches life of cirrhotic patients with refractory ascites**Toru Arano, Kazuaki Tejima, Rindo Ishii, Yuri Hanaoka, Tsuyoshi Fukumoto, Tadahiro Yamazaki, Aya Murakawa, Dai Mouri, Jun Tashiro, Jun Miwa, Masahiro Arai**

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Background: Refractory ascites for decompensated cirrhosis deteriorate the patients' quality of life. Denver peritoneovenous shunt is sometimes adopted as remedy for such cases.**Patients and methods:** Twelve patients who had undergone Denver shunt for refractory ascites associated with decompensated cirrhosis with or without hepatocellular carcinoma between April 2004 and November 2014 were analyzed retrospectively.**Results:** The median survival time after Denver shunt intervention was 168 days (range, 2 to 4033) in all cases, while it remains 98.5 days (range, 2 to 134) in four cases of patients with hepatocellular carcinoma. The median body weight decrease from Denver shunting was 10.3 kg (range 5.7 to 14.2). In 8 out of 11 patients (72.7 %) except for one who died early (2 days after intervention) due to DIC, significant improvements in the quality of life were achieved according to the interview. Complications within 1 month after Denver shunting were DIC, gastrointestinal bleeding, splenic infarction and cerebral infarction; whereas those after 1 month were shunt occlusion, infectious endocarditis, and vertebral osteomyelitis. Patients with advanced hepatocellular carcinoma were susceptible to major complications and suffered from poor outcome.**Conclusion:** Denver shunting for refractory ascites is useful in improving quality of life and also effective if candidates are selected properly. Further experiences are needed to establish the patient selection criteria.

P-0830

A female with massive ascites: a diagnostic dilemma of unknown primary**Fauzi Yusuf, Azzaki Abubakar, Siti Adewiah, M.Haris Yulis**

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Background: Ascites is pathological accumulation of fluid within abdominal, commonly associated with chronic hepatic diseases, malignant, cardiac, tuberculous, and etc. In some cases, ascites can pose a diagnostic challenge for clinicians, despite thorough and extensive work-up, the origin of this ascites remains unknown.**Case report:** In the unusual case reported, a 56-year-old woman developed severe ascites in a few months, absence of liver disease or heart failure. On physical examination, ascites and enlarged lymph glands of neck measured 5 × 5 cm, smooth surfaced, rubbery and mobile. We performed laboratory analysis and endoscopic revealed no abnormality. Abdominal CT with contrast demonstrated ascites and FNAB results chronic inflammation consistent with tuberculosis. Ascites analysis showed exudative fluid with non-specific chronic inflammation and ADA levels with normal limit. She was started tuberculosis medication. On follow up, there's no reduce clinically. Until now patients regularly paracentesis every 2 weeks.**Conclusions:** The causes of ascites include a very long list of pathological conditions that may arise primarily in several

intraperitoneal or extraperitoneal organs. We define ascites of unknown origin as the etiology and differential diagnosis of ascites therefore always remains a diagnostic dilemma.

P-0831

Variant of ascitic fluid bacterial infections in patients of liver cirrhosis**Md.Jahangir Alam Sarker¹, Md.Shahinul Alam², Moahammad Shahed Ashraf¹, Sheikh Mohammad Noor-E-Alam¹, Mohammad Faiz Ahmad Khondaker¹, Mamun-Al Mahtab², Mobin Khan²**¹Department of Hepatology, Shaheed Suhrawardy Medical College, Dhaka, Bangladesh; ²Bangabandhu Sheikh Mujib Medical University, Shahbagh, Dhaka, Bangladesh**Background:** Ascitic fluid bacterial infection in cirrhotic patients has a high morbidity and mortality. It has five variants namely, spontaneous bacterial infection (SBP), culture negative neutrocytic ascites (CNNA), secondary bacterial peritonitis, monomicrobial non neutrocytic bacterascites (MNB), polymicrobial bacterascites. Among the five variants SBP and CNNA are common ascitic fluid bacterial infection. The aim of the study was to determine the variants of ascitic fluid bacterial infection in patients of cirrhosis of liver at advanced stage (Child Pugh Class C).**Methods:** We analyzed thirty five consecutive cirrhotic patients of Child Pugh Class C with ascites admitted or attended in outpatient department of Hepatology Department, Bangabandhu Sheikh Mujib Medical University from January 2008 to December 2009. Clinical and laboratory parameters of these patients including Child Turcotte Pugh (CTP) score were recorded.**Results:** Among 35 patients 8 patients were symptomatic (22.9 %) and 27 patients were asymptomatic (77.1 %) i.e. they had no feature of ascitic fluid bacterial infection like fever, abdominal pain or tenderness. Out of 27 asymptomatic patients 6 had ascitic fluid bacterial infection i.e. 22.22 % and out of 8 symptomatic patients 2 had ascitic fluid bacterial infection i.e. 25 %. All the patients were of CNNA (culture negative neutrocytic ascites) variant.**Conclusion:** Asymptomatic ascitic fluid bacterial infection is common in cirrhotic patients.

P-0832

Minimal hepatic encephalopathy among patients with cirrhosis of liver: what is the burden?**Mohammad Golam Azam, Md. Nuruzzaman, Tareq Mahmud Bhuiyan, Abdullah Al Mamoon, Indrajit Kumar Datta, Md. Anisur Rahman**

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Background: Identifying patients with minimal hepatic encephalopathy (MHE) among cirrhotic patients may help in the management to avoid further worse consequences. This study was carried out to find out the frequency and clinical presentation of MHE in a tertiary care hospital in Dhaka, Bangladesh.**Methods:** This work was done at the department of gastrointestinal, hepatobiliary and pancreatic disorders (GHPD) at BIRDEM General Hospital from August 2013 to July 2014. Demographic, clinical and

biochemical parameters along with cognitive functions were evaluated. MHE among study subjects were screened by psychometric tests like number connection test-A (NCT-A) and digit symbol test (DST). **Results:** Eighty-five patients with cirrhosis of liver were included. Of them, 52(60.1 %) were male. Age mean \pm SD was 55.7 ± 10.3 years. Aetiology of cirrhosis was non-B non-C 43(50.6 %), hepatitis B virus 37(43.5) and hepatitis C virus 5(5.4 %). Child Pugh Score (CPS) B was 17(20.0 %) and C was 68(80 %). Frequency of MHE was 55(64.7 %) which varied significantly according to CPS; with CPS B, MHE present in 6(10.9 %) cases and in CPS C, MHE present in 49(89.0 %) cases ($P = 0.005$). There were significant differences between MHE positive and negative groups in terms of mean platelet count/cmm (83276.36 ± 33133.52 vs 108756.66 ± 73797.32 , $P = 0.03$), mean serum ammonia level micromol/L (71.04 ± 12.11 vs 40.21 ± 3.64 , $P = 0.01$), prothrombin time seconds (17.62 ± 2.72 vs 16.09 ± 2.60), serum albumin gm/L (24.17 ± 14.82 vs 27.65 ± 5.74 , $P = 0.004$).

Conclusion: MHE is quite prevalent among cirrhotic patients. Thus, cirrhotic patients with poor liver function should be tested for MHE and appropriate measures should be taken to combat MHE related morbidity and mortality.

P-0833

Assessment of minimal hepatic encephalopathy in Mongolia

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Background: Minimal hepatic encephalopathy (MHE) is characterized by relatively mild neurocognitive impairments, and occurs in a substantial percentage of patients with liver disease. The presence of MHE is associated with a significant compromise of quality of life, is predictive of the onset of overt hepatic encephalopathy and is associated with a poorer prognosis for outcome. Reaching a diagnosis of MHE can be difficult due to lack of a gold standard test. Diagnosis of MHE is still controversial, especially when it comes to establish criteria and reliable diagnostic tests applied in clinical practice. A variety of approaches have been used in efforts to detect MHE, including neuropsychological and neurophysiological tools.

Methods: A case control study at GI centre of First Central Hospital of Mongolia and Reflex hospital in 2014–2015 was carried. There 30 patients with cirrhosis were diagnosed on the basis of biochemical, ultrasonographic and endoscopic findings and 30 healthy for control group. All the participants were tested for MHE using PHES and recorded median nerve somatosensory evoked potentials (SSEP). Statistical analysis and frequencies were calculated with SPSS17.

Results: In 27 patients with cirrhosis showed up more than 2 SD in more than 2 tests. The latency of SSEP of the cirrhotic patients is in the normal limit and the conduction time is not prolonged.

Conclusion: The sensitivity of PHES is 90 %. The latency of SSEP of the cirrhotic patients has ($P < 0.05$) differences from the control group.

P-0834

Combination of lactulose, rifaximin and LOLA in treatment of minimal hepatic encephalopathy

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Background: Minimal hepatic encephalopathy (MHE) is observed in 84 % of patients with liver cirrhosis without the presence of overt HE. It adversely affects health-related quality of life (HRQOL).

Aim: To compare lactulose, rifaximin, L-Ornithine L-Aspartate (LoLa), their combination on MHE treatment and HRQOL.

Methods: After screening for MHE, 126 patients with MHE were assigned for the following treatment regimens; 30-60 ml lactulose twice daily ($n = 31$), 200 mg rifaximin thrice daily ($n = 32$), 6 g LoLa thrice daily ($n = 32$), and combined therapy ($n = 31$). All patients were assessed by critical flicker frequency (CFF), number connection test (NCT), serial dotting test (SDT), ammonia and sickness impact profile (SIP) questionnaire at baseline and two consecutive months.

Results: By repeated measure ANOVA test, there was favorable treatment induced changes in all groups concerning the three consecutive values of CFF (36.6 vs. 31.33 vs. 38.09 Hz; $p = 0.001$ except LoLa; $p = 0.167$), NCT(-2.5 vs. -1.15 vs. -0.9 SD; $p = 0.001$), SDT (-2.6 vs. -1.27 vs. -1.07 SD; $p = 0.001$), ammonia (85.75 vs. 76.93 vs. 69.65 & 60; mmol/l; $p = 0.001$ except rifaximin; $p = 0.50$) and SIP questionnaire score (24.75 vs. 16.1 vs. 16.31; $p = 0.001$). The overall comparison of all groups was insignificant (p it& 0.05). The predictors of MHE were age (odds = 1.069), bilirubin (odds = 5.254), albumin (odds = 0.163), INR (odds = 73.816) and Child-Pugh score (odds = 2.459).

Conclusion: Lactulose, rifaximin, LoLa, their combination are the same on MHE treatment and HRQOL.

P-0835

Minimal hepatic encephalopathy diagnosed with Neurophysiological test in cirrhotic patients

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Aims: Minimal hepatic encephalopathy: MHE reduces the QOL. The aim was to investigate the detection rate of abnormality in Neurophysiological tests:NP tests, the number of tests with abnormal values, and the incidence of MHE in cirrhotics according to Pugh grades and with/without the past history of OHE. Method NP tests were performed in 188 cirrhotics. NP test consists of 8 subtests. MHE was diagnosed when two or more tests gave abnormal values.

Results: Abnormal rates in 6 of 8 tests in the cirrhotics increased according to Pugh grade. The number of the abnormal tests were 1.7, 2.5, 3.3, in Pugh A, B, C, respectively and significantly increased

according to Pugh grade. There was no significant difference in the abnormal rate of each test between with and without the past history of OHE. No difference in the number of the abnormal tests was found between both groups. MHE was diagnosed in 58 % of 188 cirrhotic patients. The incidence of MHE increased according to Pugh grade, Pugh A; 46 %, B; 54 %, C; 78 %. The percentages of MHE were 57 and 61 % in patients without and with the past history of OHE. There was no significant difference between them.

Conclusions: In the present study, abnormal rates in 6 of 8 tests, the number of tests with abnormal values and the incidence of MHE increased according to Pugh grade, not the past history of OHE. From these data, MHE depended on the severity of cirrhosis but not the presence of the past history of OHE.

P-0836

Correlation between character of portal blood flow and post-tips incidence of hepatic encephalopathy

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Background: Hepatic encephalopathy (HE) is one of the major complications that follow transjugular intrahepatic portosystemic shunt(TIPS) operations in patients with different complications of portal hypertension as refractory ascites, refractory hydrothorax, bleeding varices and hepatorenal syndrome. The aim of this study was to clarify predisposing factors for post-TIPS incidence of HE in relations to Pre-TIPS hemodynamics.

Patients and methods: Fifty patients were enrolled in this study most of them with Child score A (4) and B (44). Patients were assessed by Doppler ultrasound for the flow inside the portal vein and divided into two groups: group 1; 31 patients with hepatopetal flow and group 2; 19 patients with hepatofugal flow, then TIPS was performed and patients were followed for 1 month for development of HE.

Results: There were no significant differences in multiple variables as age, gender, weight, etiology of liver disease and indication for TIPS. The incidence of HE post-TIPS was observed more in group 1(29 %) more than group 2(10 %), also it was closely related to Child Score and Pre-TIPS incidence of HE.

Conclusion: Post-TIPS incidence of HE is closely related to Pre-TIPS flow in the portal vein.

Keywords: Portal hypertension, Hepatic encephalopathy, Doppler ultrasound, TIPS

P-0837

Presence of anemia predicts advanced grade at presentation in patients with hepatic encephalopathy

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Objective: The objective of our study was to assess the impact of anemia on HE grade at presentation.

Methods: Consecutive patients of HE admitted in the medical wards of Mayo Hospital, Lahore during March 2010 and May 2010 were enrolled in the study. HE grade at presentation was assessed by using West-Haven Criteria. Complete blood count, bleeding profile, liver function tests and ultrasound was done in emergency at presentation. Anemia was defined as hemoglobin level less than 12 g/dl. Univariate and multivariate logistic regression analysis was done to assess the impact of anemia on hepatic encephalopathy grade at presentation. P value <0.05 was considered significant.

Results: 61 patients were included in the study. 20 % patients were in grade 1 HE; 20 % in grade 2; 39 % in grade 3; and 21 % in grade 4 HE. Advanced grade HE was defined as HE grade >2. On univariate analysis prothrombin time >15 s, diabetes, esophageal varices on endoscopy, and anemia were significant predictors of advanced grade HE (p values: 0.048, 0.048, 0.039, 0.037). Hypoalbuminemia was less common in advanced grade HE patients (p, 0.004). Child Pugh Score and MELD Score had no relation with HE grade at presentation. All the significant factors in univariate analysis were included in the multivariate logistic regression model. Only anemia was significant predictor of advanced grade HE in multivariate analysis (p, 0.018).

Conclusion: Sixty percent of HE patients present with advanced grade. Anemia is associated with advanced HE grade at presentation.

P-0838

A summary of using levocarnitine with liver cirrhosis in our hospital

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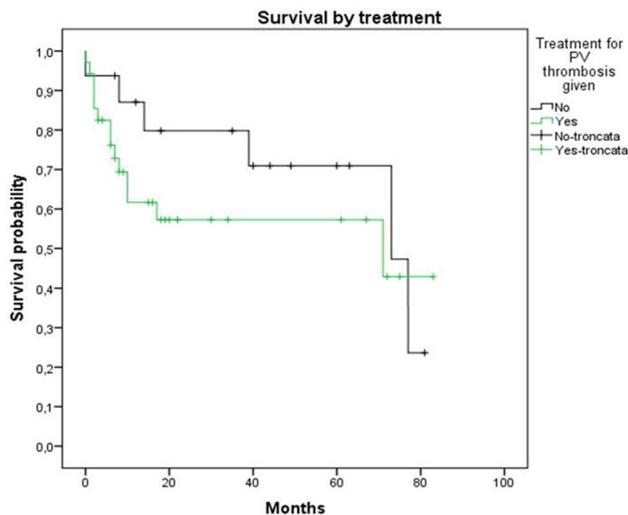
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Aim: To evaluate the effect of levocarnitine with liver cirrhosis.

Method: We use levocarnitine 14 patients (10 are men, 4 are women) in our hospital. The average age of them is 64.7 (37–77), Child-Pugh scores are 4 in score B and 10 in score C. The etiologies are 5 are HCV, 4 are alcoholic liver cirrhosis, 3 are non-alcoholic liver cirrhosis, a PBC patient and a Wilson disease patient. The 8 patients have HCC. We analysis their clinical courses retrospectively.

Result: We use them median 1157 (600–3000) mg/day levocarnitine. We can be change for the better 8 patients. Their serum ammonia levels are decreased significantly. We estimate a patient can avoid liver transplantation.

Conclusion: The levocarnitine tend to have higher effect in patients decreasing serum ammonia level. On the other hand, we have experiences same stabilization of symptoms with changing no serum ammonia level. The decision of continue levocarnitine may need to assess symptoms carefully as well as labo data.



P-0839

Comparing the TEG with conventional coagulation tests in cirrhotics undergoing invasive procedures

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Background: Coagulation cascade in patients with cirrhosis is delicately balanced between deficiency of anti and procoagulant factors. Conventional tests for coagulation only assess procoagulant factors and are poor predictors of bleeding risk. In spite of this knowledge they are routinely used prior to invasive procedures and attempts are made to correct these abnormalities before invasive procedures. These practices are not supported by the evidence and they are also harmful to the patients.

Methods: In this prospective study 95 patients with cirrhosis of liver who are undergoing invasive procedures were included. Conventional coagulation tests (CCT), thromboplastin generation time (TGT) and thromboelastography (TEG) were done in all patients and they were observed for post procedural bleeding. None of the patient received prophylactic transfusion before the procedure.

Results: 95 patients of cirrhosis (mean age of 52 + 12 years, 74 % males, 22, 31 and 42 patients were in child class A, B and C respectively. Median INR was 1.52 and median platelet count of 1.0 lakh/cumm. All patients underwent invasive procedures (liver biopsy, central line insertions, dental extraction, hemiarthroplasty, hepatic angiography, TIPSS, TACE) were enrolled in the study. TEG was normal in all patients. Only one patient developed post procedural bleeding (who was having sepsis and acute kidney injury) and was given blood transfusion.

Conclusion: Though CCT were abnormal in patients of cirrhosis, they do not predict bleeding risk. TEG is useful in patients with cirrhosis of liver, undergoing invasive procedures to assess bleeding in place of CCT prevents erroneous prophylactic transfusions.

P-0840

Correlation of hepatic cirrhosis with severity of bone changes measured by BMD

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Aim: Metabolic bone disease is underestimated complication in liver cirrhosis. Prevalence and presentation of it's in chronic liver diseases have been poorly described except cholestatic liver diseases. This study aims at evaluating prevalence and degree of bone changes in cirrhosis and correlate severity of hepatic cirrhosis with severity of bone changes.

Methods: Bone mineral density (BMD) was studied using dual energy X-ray absorptiometry (DEXA) in 60 subjects of 15-45 years. 30 had liver cirrhosis and rest of 30 were controls without any chronic disease. Diagnosis of cirrhosis was based on clinical, biochemical, and ultrasonographic findings. Patients with renal impairment, cholestatic liver disease, chronic lung disease and prolonged bed ridden patients or deformity of spine, pelvis or wrist were excluded from the study. BMD results were correlated with Child-Paugh score.

Results: Eighteen (60 %) patients had decreased BMD. Of which 15 (50 %) patients had osteopenia and 3 (10 %) patients had osteoporosis. No correlation was found between T-score and Child-Paugh score ($p = 0.236$). Significant correlations were present between BMD and serum bilirubin and albumin ($p = 0.000$).

Conclusion: Liver cirrhosis is risk factor for development of bone loss and there is high prevalence of BMD disorders in cirrhotics. Severity of bone loss is not related to severity of liver disease. Hyperbilirubinemia and low serum albumin are contributing factors to altered bone mineral density in cirrhotics.

P-0841

Serial assessment of fibrosis regression using Fibroscan in CHB patients receiving antiviral therapy

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Background: Performing liver biopsy to assess the regression of liver fibrosis in patients receiving antiviral therapy is impractical. We aimed to assess fibrosis regression by serial liver stiffness (LS) measurement using Fibroscan® during antiviral treatment, and to investigate the predictors for fibrosis regression after 5 years.

Methods: We prospectively enrolled chronic hepatitis B (CHB) patients who underwent LS measurement and liver biopsy before starting nucleot(s)ide analogues and showed histologically advanced fibrosis with a high viral loads [HBV DNA >2000 IU/mL] and ALT <2 ULN. Annual LS measurement for 5 years during antiviral treatment was performed. Fibrosis regression was defined as LS value <7.2 kPa.

Results: A total of 120 patients received antiviral treatment and annual LS measurement for 5 years. At baseline, the median LS value was 12.1dB/m, and 102(85.0 %) patients showed cirrhosis on liver biopsy. During 5 years of antiviral treatment, the median LS value significantly decreased as years go by (10.3 dB/m at Year 1; 9.0 dB/m at Year 2; 8.6 dB/m at Year 3; 7.3 dB/m at Year 4; and 6.9 dB/m at Year 5; $P < 0.001$). Fibrosis regression was identified in 63(52.5 %) at Year 5. Multivariate analysis showed baseline LS value as a predictor for fibrosis regression ($P < 0.001$; HR, 0.939; 95 % CI, 0.879–1.003) at Year 5.

Conclusion: In CHB patients with advanced fibrosis receiving antiviral treatment, the annual LS measurement revealed that fibrosis regression slows down but continues during treatment. Less amount of fibrotic burden at baseline represented as lower LS value is an independent predictor for fibrosis regression at Year 5.

P-0842

Hepatorenal syndrome: response to terlipressin and albumin and its determinants

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Objective: To determine the efficacy of terlipressin and albumin in improving renal functions in patient with hepatorenal syndrome (HRS) and to identify factors determinant of response to this treatment.

Study design: Experimental design interventional cohort study Place of study: Department of Gastroenterology, Doctors Hospital & Medical Center from July 2008 till 2015.

Patients and methods: Patients of liver cirrhosis and ascites with HRS type I were treated with intravenous albumin and incremental dosage of terlipressin based on response with maximum possible dose of 12 mg/day. Decline of creatinine <1.5 mg/dl was defined as complete response. Data was analyzed to identify factors associated with response to therapy via linear regression analysis.

Results: Twenty four patients were included with male to female ratio 3.8/1(19/5) and mean age 53.3 (± 10.06). Hepatitis C was responsible for liver cirrhosis in 21(87.5 %) patients and hepatocellular carcinoma was present in 10 (41.75 %) patients. Complete response in HRS to terlipressin/albumin was seen in 14 (58.3 %) patients, 7 (29.2 %) achieved partial response with > 25 % creatinine decline while 3 (12.5 %) had no response. Lower serum creatinine at diagnosis (P value 0.003), absence of hyperkalemia (p value 0.005) and absence of portal vein thrombosis (p value 0.05) are associated with response to treatment in HRS. Baseline serum creatinine (p value 0.003) was sole independent predictor of response to therapy in multivariate analysis.

Conclusion: Terlipressin along with albumin is an effective treatment for HRS type I. Patients with lower baseline serum creatinine are more likely to respond to this therapy.

P-0843

Endothelial dysfunction in cirrhosis and hepatorenal syndrome

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Hepatorenal syndrome (HRS) is a renal failure seen on advanced liver disease with no defined reasons. Aim of this study is to enlighten the role of endothelial dysfunction on its pathogenesis. In total, 42 patients of four groups (9 with HRS, 12 with decompensated and 11 with compensated cirrhosis and 10 with prerenal acute renal failure) and 10 healthy volunteers were examined. Endothelial dysfunction was evaluated either by biochemical parameters (Nitric Oxide (NO), Asymmetric Dimethyl Arginine (ADMA), Endothelin-1 (ET-1)) and Flow Mediated Dilatation (FMD) technique. There was a positive correlation between liver disease stage and biochemical parameters mentioned above. As severity of liver disease increases so as FMD measurement does. FMD measurements were higher for patients than controls. Positive strong relationship between creatinine and NO levels for liver disease patients was established. Similarly, for liver disease patients, this study reveals positive strong relationship between creatinine and ADMA. There was a negative strong relationship between GFR with NO and ADMA. Furthermore there was a positive strong relationship between FMD and creatinine levels and CPT score. In contrast a negative strong relationship between FMD and GFR levels were observed. This study also displayed a positive strong relationship between FMD and CPT score in HRS patients. In conclusion, endothelial dysfunction plays a role on HRS pathogenesis. Levels of endothelial dysfunction biochemical parameters may be used for the differential diagnosis of renal failure in cirrhosis. This lethal complication may be treated by new modalities capable for decreasing the levels of NO, ET-1 and ADMA.

P-0844

Relationship of child-pugh and meld scores with in-hospital mortality in decompensated cirrhosis

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Background and aims: Child-Pugh (CP) and MELD scores are important prognostic factors for cirrhotic patients. High CP and MELD scores are associated with increased short-term and long-term mortality. The aim of our study was to evaluate the relation between in-hospital mortality, and CP and MELD scores.

Methods: Consecutive patients of decompensated cirrhosis presenting at Mayo hospital, Lahore were included in the study. Patients were interviewed at bed after obtaining informed consent. Data was collected regarding basic demographic details, co-morbid conditions, etiology of cirrhosis. Laboratory investigations at presentation were noted and CP and MELD scores were calculated. Patients with CP Class A and B were categorized as having mild disease and CP Class C as having severe disease. MELD score was categorized as <15 and >15. Patients were followed up till in-hospital death or discharge from hospital.

Results: A total of 150 patients were included in the study; ninety (60 %) of them were male. Mean age of the patients was 52.9 years (SD = 12.6). Eighteen (12 %) patients died in hospital. Both the CP Class and MELD category had no relation with in-hospital mortality ($p = 0.155, 0.259$ respectively). Etiology of cirrhosis, presence of comorbid conditions (diabetes and hypertension), thrombocytopenia, hyponatremia, hyperkalemia and raised blood urea levels were also not associated with in-hospital mortality. The only predictor of in-hospital death was raised serum creatinine ($p = 0.001$).

Conclusions: Child-Pugh and MELD scores were not related to in-hospital death in our study. The only predictor of in-hospital death was raised serum creatinine.

P-0845

Prevalence and predictors of thrombocytopenia in advanced liver disease

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Objective: To study clinical, laboratory and demographic predictors of thrombocytopenia in advanced liver disease.

Methods: 248 patients with decompensated cirrhosis (DC) (age range: 30-75 years; majority with chronic C hepatitis (78 %)) were prospectively analyzed. The platelet count with cut-off value of $<150,000/\mu\text{L}$ was taken as thrombocytopenia. Patients with and without thrombocytopenia were correlated with patients' characteristics, such as demographics, prevalent extra hepatic diseases, therapeutic interventions (endoscopy, band ligation and interferon therapy), clinical signs and laboratory variables.

Results: 248 patients showed following distribution according to CP classification: A; 7 %; B; 30 % and C; 63 %. Hepatitis C (83 %, $P = 0.009$) demonstrated strong correlation but hepatitis B infection and alcohol failed to show any significant association with thrombocytopenia ($P > 0.05$). People with splenomegaly (73 %, $p = 0.000$) and elevated ALT levels (>35 IU) were more prevalent in thrombocytopenia group (96 %, $P = 0.019$). Deranged clotting parameters PT (>15 sec, 96 %, $p = 0.001$) and aPTT (>35 s, 86 %, $p = 0.023$) were strongly associated with thrombocytopenia. However no statistically significant association was observed between demographic variables, MELD score, CP classification, extra-hepatic diseases, therapeutic interventions and other clinical signs and laboratory tests ($p > 0.05$ for all).

Conclusion: Hepatitis C infection is an independent predictor of thrombocytopenia in DC. Combination of splenomegaly, elevated ALT, deranged clotting parameters can predict thrombocytopenia in advanced liver disease.

P-0846

Left ventricular dysfunction among cirrhotic with beta blocker therapy

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Introduction: Liver cirrhosis remains the 12th leading cause of death worldwide. Cirrhosis has been associated with decreased systemic vascular resistance, increased cardiac output and abnormal myocardial contractile function leading to diastolic dysfunction. The objective of this study is to evaluate cardiac dysfunction in cirrhotic patients with beta blockers by conventional 2D echocardiography and Doppler imaging.

Methodology: This is a retrospective study of patients diagnosed with cirrhosis. Demographics, clinical and laboratory parameters were recorded. The severity of liver cirrhosis was graded using Child Pugh MELD and MELD-Na scores. Statistical test used were T-test, chi-square test and logistic regression.

Results: Sixty-eight cirrhotics patients were identified. Majority were males with a mean age of 66.7 years. Mean MELD, MELD Na and Child-Pugh's score were 13.6, 17.07 and 8.21 respectively. Mean ejection fraction was 65.4 %. The mean LV, LA, RA, RV dimensions (in mm) were 47.99, 33.47, 28.76 and 27.47, respectively. The use of beta blockers has no significant effect in preservation of ejection fraction ($p = 0.246$) but has shown protective effect in terms of prevention of left ventricular diastolic dysfunction ($p = 0.02$).

Conclusion: Based on the results of this study, beta blockers may have protective effect in the prevention of Left ventricular dysfunction among cirrhotic patients.

P-0847

Predictive factors for therapeutic effects of tolvaptan to patients with refractory ascites

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Background: Severe ascites and edema will often worsen performance status of patients with decompensated cirrhosis. Furthermore such severe ascites is refractory to treatment with diuretic drugs. Tolvaptan (TLV), which is a vasopressin V2-receptor antagonist, was developed to treat with the fluid collection for patients with liver cirrhosis in Japan. In this study, we evaluated validity of TLV and determined prediction factors for its therapeutic effects.

Methods: Twenty patients treated with TLV due to the refractory ascites were enrolled from October 2013 to March 2014 in the Showa University Hospital. Correlation of blood, biochemical and urinary parameters with changes of body weight was analyzed before and after the treatment. All the patients had undertaken the other diuretic medicines (furosemide and spironolactone) at the beginning of TLV, and were initially treated with 7.5 mg per day of TLV.

Results: In baseline characteristics, the median age of the objectives was 70, and gender of 11 patients (55 %) was male. The etiology of cirrhosis was revealed as HCV infection (4 cases), alcohol consumption (7) and others (9). We estimated as "effective" of TLV for patients whose body weight was decreased more than 2 kg per 2 weeks. Sixteen of 20 patients (80 %) were classified into the "effective" group. In comparing the "effective" group with the "non-effective" group, the serum level of creatinine was significantly lower in the "effective" group ($p = 0.03$).

Conclusions: Serum level of creatinine is one of the predictive factors for the therapeutic effects of TLV on patients with refractory ascites.

P-0848

Tolvaptan reduces refractory ascites in patients with advanced liver cirrhosis.**Rika Aso, Yoshihiro Suzuki, Michihiko Tsunoda, Hiroaki Miyazawa, Rika Shibuya, Toshiki Sugawara, Takashi Hayasaka, Naomi Yoshida, Tomohiko Orii, Masanori Aoki**

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Aim: The aim of this study is to evaluate the therapeutic efficacy of tolvaptan in patients with advanced liver cirrhosis and obvious ascites.**Methods:** The subjects were 17 patients with liver cirrhosis who had been receiving combinations of loop diuretics and anti-aldosterone agents at our hospital between 2013 and 2014. All patients received 7.5 mg of tolvaptan, administered orally once daily. Body weight, urine volume, serum sodium, and creatinine concentration were measured.**Results:** The 17 patients had a mean age of 65.6 years (range, 46–83). The etiology of cirrhosis was hepatitis B for one patient, hepatitis C for seven, non-B, non-C hepatitis for 2, and alcoholism for seven. Three patients were Child-Pugh classification B and 14 were C. The mean bodyweights were 61.6 (range 42.6–82.4; n = 15), 58.6 (range, 40.6–68.8; n = 14), 57.7 kg (range 38.2–67.6; n = 12) on day 0, 3 and 7, respectively. The mean urine volume were 1611.3 (range 720–4300; n = 16), 2649.4 (range, 250–5500; n = 16), 2304.0 ml (range 150–6900; n = 115) on day 0, 3 and 7, respectively. Serum sodium concentration and creatinine concentration were did not change significantly. Nine patients continued tolvaptan during this period, one patient was able to quit tolvaptan because his ascites was well controlled. However, seven patients died for liver decompensation. Extent of liver damage and history of hepatocellular carcinoma were relevant to the prognosis of these patients.**Conclusion:** The efficacy and tolerability of tolvaptan suggest that it could be an important option for treatment of refractory ascites with advanced liver disease.

P-0849

Hepatic ascites treatment with novel diuretic: vasopressin V2 receptor antagonist (Tolvaptan)**Hideo Yoshida, Hiroyoshi Taniguchi, Ryo Nakata**

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Background: Medical treatment of hepatic ascites has been based on furosemide and spironolactone for long time. Recently, novel diuretics of Tolvaptan become available in Japan.**Methods:** Tolvaptan was administered for 32 patients with refractory hepatic ascites between September 2013 and August 2015. Patients received 3.75 or 7.5 mg/day of Tolvaptan orally. Demographic data, body weight, daily urine volume, serum creatinine, serum sodium concentration, and liver numbers were collected.**Results:** In 32 patients, 23 were male and 9 were female. Age of patients ranged between 44 and 83 (median 68 yo). Etiology of liver disease was as follows: HCV 16, Alcoholic liver disease 8, Others 8. Child Pugh score ranged between 8 and 13 (median 10). Fourteen were Child B and 18 were Child C. Urine volume increased $+672 \pm 682$, $+716 \pm 827$, and $+305 \pm 776$ in day 1, 7, and 14 respectively. Fifty seven percent of patients had BW loss more than 1 kg in 2 weeks. No severe adverse effect including acute elevation of

serum sodium level was seen. Seven patients stopped taking Tolvaptan because of no effect (two patients), general malaise, encephalopathy, pneumonia, dehydration and of recovery from ascites.

Conclusions: Tolvaptan was safely administered for the treatment of hepatic ascites. This novel diuretic provide alternative option for the treatment of end stage liver disease.

P-0850

Serum M2BPGi and HVPG are useful factors predicting the effect of tolvaptan on hepatic edema**Norio Itokawa¹, Masanori Atsukawa¹, Tomomi Okubo¹, Taeng Arai¹, Ai Nakagawa¹, Chisa Kondo², Katsuhiko Iwakiri²**¹Division of Gastroenterology, Department of Internal Medicine, Nippon Medical School Chiba Hokusoh Hospital, Chiba, Japan;²Division of Gastroenterology and Hepatology, Nippon Medical School, Bunkyo-ku, Tokyo, Japan**Aim:** Previous studies have been reported that tolvaptan is effective on hepatic edema. The aim of prospective study was to clarify which factors of baseline could influence the effect of tolvaptan in cirrhotic patients with conventional diuretic-resistant hepatic edema.**Method:** Forty patients with hepatic edema was not improved with conventional diuretics, so tolvaptan was orally administered at a dosage of 7.5 mg once a day more than 7 days. Hepatic venous pressure gradient (HVPG) was measured in 28 patients. This study was approved by the Ethics Review Board of Nippon Medical School Chiba Hokusoh Hospital.**Result:** Patients consisted of 26 males (65 %), with the median age of 65 years (40–82 years). The etiology of liver diseases was hepatitis C for 15 patients, hepatitis B for 3, alcoholic hepatitis for 15 and others for 3. The median HVPG value was 227.5 (105–305) mmH₂O. Nineteen (47.5 %) were determined as responder. The median decrease in body weight was -1.3 kg (-6.1 to 1.7 kg), which was significantly lower than the baseline. A comparison of the therapeutic effect at baseline factors, M2BPGi and HVPG were extracted as significant factors. Patients with low M2BPGi levels were more effective than in those with high levels ($p = 0.032$), and patients with low HVPG were also more effective to tolvaptan than in those with high HVPG ($p = 0.030$).**Conclusion:** Present study suggested that M2BPGi and HVPG at the baseline are useful factors predicting the effect of tolvaptan on hepatic edema in patients with decompensated cirrhosis.

P-0851

Portosystemic gradient is significantly different on day 5 compared to immediately post-TIPS**Pik Eu Chang¹, Kevin Teh¹, Apoorva Gogna², Chow Wei Too², Farah Irani², Richard Lo², Bien Soo Tan², Kiang Hiong Tay²**¹Department of Gastroenterology and Hepatology, Singapore General Hospital, Singapore, Singapore; ²Department of Interventional Radiology, Singapore General Hospital, Singapore, Singapore**Background:** The portosystemic gradient (PSG) after TIPS insertion is an important hemodynamic indicator. Current guidelines recommend reduction of post-TIPS PSG to <12 mmHg to prevent recurrence of variceal bleeding. However, the optimal timing for measurement of post-TIPS PSG is unclear. In this study we aim to

explore if there is any difference between the PSG measured immediately after TIPS insertion (PSG_D0) compared with PSG measured 5 days later (PSG_D5). We hypothesize that this difference is due to equilibration of atrial pressures that occurs due to cardiac remodelling.

Methods: Prospective, single-center study of 21 consecutive patients who had TIPS insertion for treatment of portal hypertensive complications. PSG was measured at the end of TIPS insertion and was repeated 5 ± 2 days later. Differences were compared using paired sample T-test.

Results: Mean age was 58.7 ± 12.0 years; median MELD score was 10 (range 7–24) with 33/62/5 % Child A/B/C respectively. Indication for TIPS was for variceal bleeding (57 %) and refractory ascites (43 %). Mean pre-TIPS HVPg was 16.1 ± 5.5 mmHg. PSG_D5 was significantly higher compared to PSG_D0 (7.6 ± 3.6 vs. 5.8 ± 2.7 mmHg, $p = 0.024$) despite no significant change in portal vein pressure. Baseline mean right atrial (RA) pressure (7.3 ± 6.1 mmHg) increased to 10.5 ± 3.3 mmHg immediately after TIPS insertion but reduced back to a mean of 7.3 ± 7.4 mmHg at day 5 ± 2 post-TIPS.

Conclusion: Post-TIPS PSG measured immediately after TIPS insertion is inaccurate because the RA pressure is erroneously high due to the sudden increased pre-load. RA pressures undergo remodelling and return to baseline values within a week. Portal pressures should be repeated 5 days post-TIPS to obtain a more accurate PSG

P-0852

Role of Von Willebrand factor levels in predicting severity of cirrhosis

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Background and aims: Von Willebrand factor (vWF) a surrogate marker of endothelial dysfunction, is increased in patients with cirrhosis but the clinical significance is unclear. vWF-Ag plays an important role in primary haemostasis and development of thrombotic vascular obliteration which is a possible mechanism leading to portal hypertension. In this study we evaluated the association of serum VWF antigen (Ag) level with the severity of cirrhosis (according to Child-Pugh classification), its correlation with patients having thrombosis and size of oesophageal varices in cirrhotics.

Methods: We included 81 cirrhotic patients (Male 59, Mean Age $59 + 11$) in the study. Diagnosis of cirrhosis was made on the basis of biopsy, clinical, laboratory and imaging parameters. VWF Ag level was done using Von Willebrand Factor antigen (vWF:Ag) 96-microwell test kit from Helena biosciences Europe. The stage of cirrhosis was defined with Child-Pugh classification and MELD score. Data were analysed by using Statistical Package for the Social Sciences (SPSS) 10.0 software program.

Results: We observed there was no statistically significant difference in the VWF Ag levels with the increasing stages of cirrhosis according to Child-Pugh score, in patients with thrombosis and no thrombosis and size of oesophageal varices.

Conclusion: Our study does not show any correlation of increase in Serum VWF Ag levels in with increase severity of cirrhosis as shown in previous studies.

P-0853

Factors related to skeletal muscle volume in patients with chronic hepatitis C

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Aim: The aim of the present study was to elucidate the relationship between the skeletal muscle volume (SMV) and liver function or fibrosis in patients with chronic hepatitis C (CHC) and HCV related liver cirrhosis (CLC).

Methods: 223 patients with CHC and CLC were evaluated. The cross-sectional skeletal muscle area of the third lumbar levels in CT scan were quantified by image analysis software, and L3 skeletal muscle index (L3-SMI) (cm^2/m^2) was calculated as the area divided by the square of height. Velocity of shear wave (Vs) which is correlated with liver fibrosis was measured by acoustic radiation force impulse.

Results: Male/female = 129/94, age 71 (65–77) years, body mass index (BMI) 22.2 (20.0–24.6), chronic hepatitis (CH)/Child-Pugh (CP)-A/B/C = 31/94/63/35. In male, L3-SMI was significantly correlated with age ($r = -0.313$), body weight (BW) ($r = 0.545$), BMI ($r = 0.682$), and body surface area (BSA) ($r = 0.449$), and significantly lower in the patients with liver cancer ($p < 0.001$) or LC ($p = 0.001$) compared with those with CH, but there was no difference among CP-classifications. In female, L3-SMI was significantly correlated with age ($r = -0.210$), BW ($r = 0.468$), BMI ($r = 0.578$), BSA ($r = 0.367$), Vs ($r = 0.368$), T-Bil ($r = 0.241$), eGFR ($r = -0.221$). BMI and the presence of LC in male, and BMI in female were selected in multiple regression analysis, respectively. L3-SMI was not correlated with platelet count, APRI, and FIB-4 index.

Conclusion: These results suggest that SMV reduction is associated with the development of CLC in male. SMV reduction might not be detected because it is too small in female compared with male.

P-0854

Measurement of muscle mass using CT is useful to diagnose sarcopenia in patients with cirrhosis

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Objectives: Asian Working Group for Sarcopenia (AWGS) recommended cutoff values for muscle mass measurements by using bioimpedance analysis (BIA) to diagnose sarcopenia. However, diagnostic cutoff value for measurement of muscle mass using CT images in Asian populations including Japanese is not determined. The present study investigated the cutoff value for skeletal muscle mass index using CT to diagnose sarcopenia in Japanese LC patients.

Methods: We enrolled 88 LC patients underwent BIA and CT scan between November 2013 and February 2015. We diagnosed the

presence of sarcopenia using BIA according to AWGS criteria. On the other hand, the cross-sectional area of skeletal muscles was measured at the level of the third lumbar vertebra on the scan. The skeletal muscle area was then normalized by the height squared to give the skeletal muscle index using CT (SMI-CT). The best cutoff value of SMI-CT to estimate sarcopenia was suggested through the ROC curve.

Results: The prevalence of sarcopenia diagnosed by BIA was 28.8 % in men and 40.9 % in women. The area under the curve (AUC) of the SMI-CT was 0.856 with 95 % CI of 0.752–0.959 for men, and 0.752 with 95 % CI of 0.542–0.963 for women. Optimal cutoff value was 46.2 cm²/m² for men, and 37.4 cm²/m² for women. Sensitivity and specificity for sarcopenia were 78.9 % and 78.7 % for men, 55.6 and 92.3 % for women, respectively.

Conclusions: The presence of sarcopenia could be detected using CT with high accuracy. Obtained cutoff values of CT-SMI may be useful to diagnose sarcopenia in Japanese population.

P-0855

Effects of nutritional therapy on complications and outcome in cirrhosis—randomized trial

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Background and aims: Malnutrition associated with increases morbidity and mortality in patients with liver cirrhosis. There is lack of data on nutritional management in patients of cirrhosis. We performed a randomized controlled trial to assess the effects of nutritional therapy on complications and outcome in patients of cirrhosis with minimal hepatic encephalopathy (MHE).

Methods: In a tertiary care center in New Delhi, India, patients with cirrhosis with MHE were randomly assigned to groups given nutritional therapy (30–35 kcal/kg/day, 1.0–1.5 g vegetable protein/kg/day; n = 60; age 42.1 ± 10.3 years, 48 men) or no nutritional therapy (patients continued on their same diet; n = 60, age 42.4 ± 9.6 years, 47 men) for 6 months in 2014. MHE was diagnosed based on psychometry hepatic encephalopathy score (PHES). Primary endpoints were complications of cirrhosis requiring hospitalization, or death.

Results: There was no significant difference in baseline PHES (−8.12 ± 1.32 vs −8.53 ± 1.38; P = .08) scores. During the study period number of patients required hospitalization were less in nutritional therapy group (8 vs 16, P = 0.03) while mortality was comparable in both the groups (5 vs 9, P = 0.12). Overt HE developed in 10 % of patients in the nutritional therapy vs 21.7 % of the control group (P = .04). After the 6 months study period, a higher proportion of patients in the nutritional therapy group had reversal of MHE (71.1 vs 22.8 %; P = .001).

Conclusion: Nutritional therapy decreases hospitalization, effective in prevention of overt HE and treatment of MHE in patients with cirrhosis.

P-0856

Nutritional assessment in cirrhosis of liver

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Aims and objectives: To determine the prevalence of malnutrition in patients with cirrhosis using standard nutritional assessment tools and to compare nutritional differences between various etiologies of cirrhosis and different nutritional assessment techniques.

Methods: All adult patients with cirrhosis of liver with different etiologies were selected. Nutritional assessment was based on the following: anthropometry [midarm muscle circumference (MAMC)], visceral proteins, handgrip strength (HG) and subjective global assessment (SGA).

Results: Out of 150 patients with cirrhosis, 113 patients (75.3 %) were male and 37 (24.7 %) were female. The mean caloric intake was low at 16.32 ± 2.20 kcal/kg/day. The most common etiology of cirrhosis was alcohol, seen in 69 (46 %) patients. 15.3, 42.66 and 42 % patients were classified into SGA A, B and C respectively. 38, 84.7, 82 and 74.33 % patients had malnutrition by MAMC, SGA, HG and serum albumin criteria (<3.5 mg/dl) respectively. Prevalence of malnutrition by SGA, albumin and HG was significantly higher than with MAMC p < 0.05. There was no difference in malnutrition when SGA, HG or serum albumin was compared with each other. Malnutrition was not different between alcoholics and non alcoholic cirrhotics. Child C cirrhosis had statistically significant malnutrition as compared to child A and child B cirrhosis by SGA, HG and MAMC as a method of nutritional assessment.

P-0857

Serum 3-hydroxyisobutyrate as a biomarker of muscular BCAA catabolism in liver cirrhosis patients

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Introduction: In the liver cirrhosis (LC) patients, branched-chain amino acids (BCAAs) in the skeletal muscle mitochondria are catabolized to compensate energy generation and ammonia detoxification. Consequently, muscle atrophy and its accompanied symptoms would appear. In the BCAA catabolic pathway, low molecule 3-hydroxyisobutyrate (3HIB) that is an intermediate of valine leaks out from the mitochondria to extracellular fluids. Therefore, we evaluated serum 3HIB level in the LC patients using new quantitative method.

Methods: 3HIB was measured in sera collected from 32 LC patients, 48 healthy controls, and other gastrointestinal disease patients (chronic hepatitis C, ASH, NASH, Crohn's disease, ulcerative colitis, and PBC). Furthermore, 3HIB was retrospectively evaluated in conserved sera collected from a LC patient with liver failure for 5 years. Serum 3HIB level was quantified by HPLC-negative ESI-MS/MS system.

Results: Serum 3HIB level in the LC patients was significantly higher than in healthy controls and other gastrointestinal disease patients. In the LC patients, serum 3HIB level was not correlated with either serum albumin or total bilirubin levels. Serum 3HIB level in the LC patient with liver failure was markedly increased than the mean of LC in some points of the 5-year period.

Conclusion: Higher 3HIB level in the LC patients than healthy controls and other diseases shows that BCAA catabolism in the skeletal muscle is enhanced in LC. Because there were no correlations with other biochemical parameters, 3HIB is an independent biomarker for the monitoring BCAA catabolism in skeletal muscles and for the management of LC patients.

P-0858

Prediction of the response to branched-chain amino acid medication by liver stiffness in cirrhosis

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Aim: Evidence has shown the clinical benefit of branched-chain amino acid (BCAA) supplementation in cirrhosis. However, prediction of the response to the treatment is not necessarily easy. The aim was to examine the utility of liver stiffness measurement (LSM) in the nutritional management of patients with cirrhosis.

Methods: This prospective study was performed in 60 consecutive patients (64.1 ± 11.9 years, male 30, female 30) with cirrhosis defined by LSM (12.5 kilopascals [kPa]; FibroScan™, Echoscans, Paris, France). Application of oral BCAA supplementation was based on the clinical decision, and nutritional changes over a 1-year period were assessed by measuring variation in serum albumin levels (improvement, ≥0.3 g/dL; deterioration, ≤−0.3 g/dL; no change, between them).

Results: There were 15 patients with BCAA supplementation and 45 patients without. The LSM value (kPa) was higher in the former (29.7 ± 12.4) than in the latter (22.1 ± 8.4, $P = 0.040$). The response to the supplementation was improvement in 5/15 patients (33.3 %), no change in 4/15 (26.7 %), and deterioration in 6/15 (40.0 %). The area under the receiver operating characteristic curve was 0.820 to detect the nutritional improvement with 70.0 % sensitivity and 100 % specificity under the best cut-off value of 28.0 kPa. The improvement was more frequently observed in patients with LSM <28.0 kPa (5/8) than those with LSM ≥28.0 kPa (0/7, $P = 0.026$). **Conclusions:** The LSM has a role to select the potential candidate for BCAA supplementation effectively by predicting the nutritional response in cirrhosis.

P-0859

The association between hepatitis B/C infection and metabolic syndrome in northern Taiwan

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Aim: Through a cross-sectional survey, we evaluated the association between hepatitis B virus (HBV) and hepatitis C virus (HCV) infection and metabolic syndrome (MS) in northeastern coast region of Taiwan. **Method:** We conducted a community-based study from 2013 to 2014 in four Taiwanese districts. 3468 adult participants were enrolled with mean age of 57.9 years. Blood test included liver biochemistry, HBV surface antigen (HBsAg), antibody to HCV (anti-HCV) and biochemical markers related to MS. Anthropometric measures related to MS, such as waist circumference and blood pressure were also collected. Insulin resistance was defined as HOMA-IR >2.5.

Results: 459 (13.2 %) participants were positive for HBsAg and 137 (4.0 %) participants were positive for anti-HCV. Compared to HBsAg negative, HBsAg positive has significant lower prevalence of hypertriglyceridemia (≥150 mg/dl) and hypercholesterolemia (≥200 mg/dl) in persons ≥50 years of age. Compared to Anti-HCV negative, Anti-HCV positive has significant higher prevalence of MS, central obesity, low HDL (male <40 mg/dl, female <50 mg/dl), and insulin resistance; and significant lower prevalence of hypercholesterolemia, and elevated LDL (≥130 mg/dl) in persons ≥ 50 years of age. No significant difference in prevalence of high blood pressure (SBP ≥135 mmHg or DBP ≥85 mmHg) and hyperglycemia (≥100 mg/dl) was observed between positive for HBsAg or Anti-HCV or not.

Conclusions: There was no significant association between MS and HBV infection whereas HCV infection has positive association with MS, dyslipidemia and insulin resistance.

P-0860

Comparison the accuracy of clinical assessment tools to diagnose malnutrition in cirrhosis

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Introduction: Malnutrition is an underdiagnosed problem that affects mortality besides quality of life in cirrhotic patients. We compared subjective global assessment (SGA), nutrition risk screening 2002 (NRS) test, anthropometric measurements in cirrhotic patients and in control groups to assess nutritional status.

Methods: All consecutive cirrhotic patients admitted to the clinic during January-December 2013 were enrolled. Control group (control 1) that was consisted of patients without liver disease but with a chronic disease (either diabetes or cardiovascular disease or cerebrovascular diseases or neurodegenerative diseases) was enrolled. A second control group (control 2) was recruited from healthy hospital staff. All patients and control subjects were evaluated by the investigator. Subject global assessment, nutrition risk assessment tests and anthropometric measurements were done by the pre-trained study investigator.

Results: 62 patients (age = 56.9 ± 15.7, 41 male [66 %], Child-Pugh score = 7.5 ± 2.2, MELD = 13.1 ± 6.4) were enrolled. Control 1 was consisted of 64 patients (age = 63.7 ± 11.8, 33 male [51.6 %]). Patients in Control 1 had at least one chronic disease. Control 2 was consisted of 34 healthy subjects (age = 26.1 ± 2, 20 male [58.8 %]). SGA and NRS diagnosed 45 (72.6 %) and 22 (35.5 %) malnourished cases in the patient group. The patient group had lower handgrip strength ($P < 0.001$), less tricep skin fold thickness ($P = 0.001$), lower serum albumin ($P < 0.001$), lower LDL ($P < 0.001$) compared to control 1 and control 2. Despite similar weight, body fat percentage was lower in the patient group ($p < 0.001$). SGA and NRS correlated with Child ($P = 0.001$ and $P = 0.001$) and MELD scores ($P = 0.001$ and $P = 0.03$)

Conclusion: Malnutrition is a frequent complication in cirrhosis. SGA and NRS are easily applicable bedside tools to detect malnutrition in cirrhosis.

P-0861

Aspirin administration may be a therapeutic option for cirrhosis-related thrombocytopenia

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Background and aims: Cirrhosis-related thrombocytopenia is explained mainly by hypersplenism and decreased thrombopoietin production. Alternatively, cirrhosis-related thrombophilia is also suggested to contribute to thrombocytopenia via platelet overconsumption. Hence anti-thrombotic therapy may increase circulating platelets in cirrhosis patients. To elucidate effects of aspirin administration, we performed a following retrospective study.

Methods: Twenty-six patients (17 men; 73 ± 9 years) with cirrhosis-related thrombocytopenia ($<130 \times 10^9/L$), who had received aspirin as a therapy for thrombotic disorders, were selected. They were divided into three groups according to the presence or absence of splenomegaly and severe hypoalbuminemia (<3.5 g/dL). Outcomes were determined at the end of administration in cases of 0.5–6 months administration or at the nearest point from 6 month of administration in cases of >6 months administration, as either “improved” ($\geq 130 \times 10^9/L$ or $\geq 10\%$ greater than initial counts) or “unimproved”.

Results: The platelet count was “improved” in 9 patients (34.6 %) and “unimproved” in 17 patients (65.4 %). The pre-administration platelet count did not differ between them. The recovery of platelet count was closely associated with the absence of splenomegaly ($P = 0.046$). The serum albumin level tended to be higher in the improved patients than in the unimproved patients ($P = 0.059$). In contrast to the 100 % efficacy in patients without splenomegaly and severe hypoalbuminemia (4/4 improved; $P = 0.006$), all patients with splenomegaly and severe hypoalbuminemia resulted in “unimproved” (0/5 improved) and patients with splenomegaly or severe hypoalbuminemia showed intermediate outcomes (5/17 improved).

Conclusions: Aspirin administration may be expected as a therapeutic option for cirrhosis-related thrombocytopenia, especially in patients without hypersplenism and/or severe hypoalbuminemia.

P-0862

The prescription pattern of acetaminophen and NSAIDs in patients with liver cirrhosis

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Background and aims: Analgesics, well-known hepatotoxic drugs, are frequently prescribed in cirrhotic patients who are prone to drug-induced liver injury. No guidelines are available regarding the

prescription of analgesics in patients with liver cirrhosis. This large-scale population study aims to evaluate the prescription patterns of most frequently used analgesics- acetaminophen and non-steroidal anti-inflammatory drugs (NSAIDs) in cirrhotic patients.

Methods: This study population was obtained from Health Insurance Review Assessment Service (HIRA) database. We assessed the prescription pattern of acetaminophen and NSAIDs in patients with liver cirrhosis between 1 January 2012 and 31 December 2012.

Results: A total of 125,505 patients with liver cirrhosis were registered from 1 January 2012 to 31 December 2012. The study population consisted of 50,798 (40.5 %) cirrhotic patients who claimed reimbursement at least one prescription for acetaminophen or NSAIDs for 1 year follow-up period. All most all patients were compensated cirrhosis (97.5 %). NSAIDs (82.7 %) was more prescribed than acetaminophen (64.5 %). NSAIDs were more frequently prescribed in compensated cirrhotic patients than decompensated cirrhotic patients ($p = 0.0001$) while acetaminophen did not show difference. Acetaminophen tended to be more frequently prescribed by internists especially gastroenterologists compare to any other physicians in compensated or decompensated cirrhosis patients. NSAIDs was less frequently prescribed by internists compare to any other physicians ($p = 0.0001$). **Conclusions:** The prescription pattern of analgesics were different significantly among physicians in patients with liver cirrhosis. Non-gastroenterologists more frequently prescribed NSAIDs than acetaminophen. The potential harm of NSAIDs in cirrhotic patients should be emphasized in non-gastroenterologists.

P-0863

Prognostic indicators of mortality among cirrhotics admitted at the intensive care unit

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Introduction: Cirrhosis is a significant source of morbidity and mortality. The over-all prognosis for patients with cirrhosis admitted to Intensive Care Unit (ICU) remains poor. The objective of the study is to identify prognostic indicators of mortality among cirrhotic patients admitted to ICU.

Methodology: This is a retrospective study of patients diagnosed with cirrhosis admitted to ICU between June 1, 2009 to June 30, 2014. Demographics, clinical and laboratory parameters were recorded. The severity of liver disease was graded using Child Pugh and MELD-Na scores. Statistical test used were T-test and chi-square test.

Results: Fifty-one cirrhotic patients were identified. Majority of patients were male with Hepatitis B virus infection as the most common etiology of cirrhosis. The most common indication for ICU admission was hepatic encephalopathy. The overall mortality rate in the ICU was 39.22 % in which the common cause of death was multi-organ failure (45 %). Mean MELD-Na and Child Pugh scores were 44 and 11 respectively. Non-survivors used a mechanical ventilator (35.29 %) and needed early inotropic support (31.37 %) and had undergone hemodialysis (25.49 %). Laboratory findings showed that non-survivors had significantly lower venous pH and bicarbonate values than the survivors.

Conclusion: Cirrhotic patients admitted at the ICU carry a grave prognosis. Higher MELD-Na scores, lower venous pH and bicarbonate values and use of mechanical ventilator, with early inotropic support and had undergone hemodialysis have been found to be related to high mortality.

P-0864

Clostridium difficile infection in Liver Cirrhosis Patients Increased Risk of Malnutrition

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Malnutrition is common in patients with liver cirrhosis and is associated with complications like ascites, hepatic encephalopathy, infections, and poor outcomes. In several case reports, Clostridium difficile infections in liver cirrhosis increase the risk of malnutrition in liver cirrhosis. The magnitude of this problem is still unknown. The purpose of this study is to know the risk of malnutrition in liver cirrhosis patients who had C. difficile infection. This cross-sectional study was conducted in rural general hospital in 2013. The hospitalized liver cirrhosis patients, diagnosed using abdominal ultrasound, were included in this study. Faeces collected from subjects were checked for C difficile antigen and toxin using Techlab 174; C. Diff Quik Chek Complete. All patients were evaluated if they are having malnutrition using Mini Nutritional Assessment (MNA) tool. The risk of malnutrition in liver cirrhosis patients who had C difficile infection were evaluated using Chi Square analysis. Fifty five patients [age 51(16–79) year old, 63.6 % male] were included in the study. Etiology of cirrhosis was hepatitis B infection in 52.7 % patients. Prevalence of malnutrition was 27.3 %. Prevalence of C difficile infection was 12.8 %. Nutritional status in C. difficile infection group was poor as assessed by MNA (28 vs 27 %; $p < 0.24$) and serum albumin level on admission (2.35 vs 2.52 g/dl; $p < 0.04$). Prevalence of malnutrition in liver cirrhosis patients was 27.3 %. Liver cirrhosis patients with C. difficile infection had nutritional status poorer based on MNA and albumin level on admission.

P-0865

Nitric oxide: the link between spontaneous bacterial peritonitis and hepatorenal syndrome

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Aim: Ascites, spontaneous bacterial peritonitis (SBP) and hepatorenal syndrome (HRS) are common complications of liver cirrhosis. Nitric oxide (NO) is a potent vasodilating molecule with a major role in splanchnic arteriolar vasodilatation and the development of ascites and HRS. We measured NO level in serum and ascitic fluid of cirrhotic patients with or without SBP.

Methods: 64 patients with liver cirrhosis, portal hypertension and ascites were enrolled. They were divided into: Group I (fifteen patients with sterile ascites and no HRS), group II (seventeen patients with sterile ascites and HRS), group III (seventeen patients with SBP and no HRS) and group IV (fifteen patients with SBP and HRS). The mean value for NO level in ascitic fluid and serum was 57.45 ± 23.01 mmol/L and 32.90 ± 9.55 mmol/L respectively. Both demonstrated significant difference among the studied groups ($p < 0.001$). Levels were lowermost in patients with sterile ascites

and normal renal functions, they became higher in patients with isolated SBP or isolated HRS, and they were highest in patients having both SBP and HRS. Serum and ascitic fluid NO levels were positively correlated. Ascitic fluid NO level correlated positively with Child score and serum creatinine, while it correlated negatively with the mean arterial pressure.

Conclusion: NO plays a complex role between SBP and HRS in the setting of liver cirrhosis and ascites. It is probably the main precipitating factor for HRS in SBP. However, isolated HRS without SBP can by itself induce increased levels of NO.

P-0866

Combined norfloxacin and rifaximine as primary prophylaxis for spontaneous bacterial peritonitis

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Background and aim: Primary prevention of spontaneous bacterial peritonitis (SBP) is important strategy to reduce morbidity and mortality in cirrhotic patients with ascites. Efficacy and safety of combined Rifaximine and Norfloxacin as primary prophylaxis is questionable.

Patients and methods: Three hundred thirty three cirrhotic patients with high SAAG (>1.1) ascites, advanced liver disease (Child Pugh score >9), functional renal impairment (cr. >1.2 mg/dl), low serum sodium (< 130 meq/l) were included in a single blind, randomized study aimed at comparing combined Norfloxacin and Rifaximine vs. Norfloxacin or Rifaximine alone as primary prophylaxis for SBP. Both intention to treat and per-protocol efficacy analysis were done after 6 months of prophylaxis by assessment of ascetic fluid neutrophil count. Safety analysis was done for all intention to treat population.

Results: Combined Norfloxacin and Rifaximine showed superior prophylaxis by intention to treat (74.7 vs. 56.4 vs. 68.3 %, $p < 0.05$) and per protocol analysis (90.8 vs. 77.2 vs. 87.5 %, $p < 0.05$), with lower probability to develop SBP (3.8 vs. 11.5 vs. 6 %) in Norfloxacin and Rifaximine groups respectively. There was no difference among the studied groups with regard to the incidence and severity of adverse events reported.

Conclusions: The combined Norfloxacin and Rifaximine based primary prophylaxis for SBP showed higher efficacy with same safety profile when compared with Norfloxacin or Rifaximine alone.

P-0867

Clinical features of spontaneous bacterial peritonitis; ten years single center experience

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Aims: Recently, the prognosis for spontaneous bacterial peritonitis (SBP) has improved. However, domestic data of SBP is limited. We aimed to review clinical course of SBP patients during 10 years in single center.

Methods: This study enrolled patients diagnosed with SBP from year 2005 to year 2014. The medical records of these patients were reviewed.

Results: Total 95 patients were enrolled. Male patients were predominant (n = 65, 68.4 %) and mean age was 58.2 years. The most common etiology of cirrhosis was hepatitis B (n = 51, 53.7 %) and hepatocellular carcinoma patients were 46 (48.4 %). Microorganism was isolated from the ascetic fluid in 49 patients (51.6 %) and other 46 patients were culture negative neutrocytic ascites. The proportions of cultured bacteria that were Gram negative and Gram positive were 81.3 % (n = 39) and 18.7 % (n = 9), respectively. The proportions of *Escherichia coli*, *Klebsiella* species, and *Streptococcus* species were 47.9 % (n = 23), 22.9 % (n = 11), and 10.4 % (n = 5), respectively. Among *Escherichia coli*, 5 cases were ESBL positive. The most commonly used first line antibiotics was cefotaxime (n = 80, 84.2 %). Hepatorenal syndromes were developed in 18 patients (18.9 %). Prophylactic antibiotics treatment was performed only in 4 patients and SBP was recurred in 9 patients (9.5 %). When compared SBP recurrence groups with non-recurrence group, laboratory findings of their serum and ascetic fluid has no significant difference between two groups.

Conclusions: Currently, SBP patients are treated effectively. However, ESBL-producing *E. coli* is commonly detected and prophylactic antibiotics use is still limited. Further study about prophylaxis of SBP recurrence is warranted.

P-0868

Safety and efficacy of stem cell stimulating therapy in decompensated cirrhosis of liver

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Aim: Aim was to assess safety and efficacy of stem cell stimulating therapy in decompensated cirrhosis as granulocyte colony stimulating factor is known to stimulate stem cells along with other immunological factors in hosts.

Methods: Ten patients with decompensated cirrhosis were included. Informed, written consent was obtained from all. There were 5/10(50 %) males and rest females. 3/10(30 %) had hepatitis B-related, 3/10(30 %) hepatitis C-related and 4/10(40 %) had cryptogenic cirrhosis. They were given injection granulocyte colony stimulating factor (30IU) sub-cutaneously weekly for 6 weeks in addition to

standard of care. In all 12 sub-cutaneous injections (Inj. Filgrast, 30IU, Beacon Pharmaceuticals, Bangladesh) were administered.

Results: At baseline, all had decompensated cirrhosis 7/8(87.5 %) had clinical jaundice (serum bilirubin 0.8–11.4 mg/dl) (mean 5.2 mg/dl), 3/8 (37.5 %) detectable ascites (serum albumin 25–38 gm/L) (mean 29.4 gm/L). Their serum ALT was 28–73 IU/L (mean 42 IU/L), INR 1.04–3.2 (mean 1.64), serum creatinine 0.6–1.6 mg/dl (mean 1.1) and total leucocyte count (TLC) 2500–16000/cmm (mean 5200/cmm) at point of inclusion. Eight had completed treatment at data analysis without any notable adverse events. No ALT flair documented in any patient. At end of 12 weeks of treatment, 7/8(87.5 %) were alive. None had ascites. Analysis at the end of 12 weeks showed, serum bilirubin 5.2 mg/dl, serum albumin 29.4 gm/L, serum ALT 46 IU/L, INR1.57, serum creatinine 1.7 mg/dl and TLC 6100/cmm.

Conclusion: Stem cell stimulating therapy was found to be safe in decompensated cirrhosis. It however failed to show significant improvement in laboratory parameters, although absence of ascites was noteworthy achievement. Larger study with longer follow up and better technique will be needed to reach definite conclusion.

P-0869

Dermatological manifestations of chronic liver disease

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Aim and objective: The magnitude of liver diseases in Bangladesh is increasing gradually. Liver disease is one of the major issues causing mortality and morbidity both among urban and rural population affecting all age groups. Aim of this study is to observe the cutaneous manifestations of chronic liver disease (CLD).

Methods: Total 120 patients from 12 to 90 years of age of both sexes admitted in Rajshahi Medical College Hospital with the history or complaints of liver disease/disorders but manifested with skin lesions were studied (following certain inclusion and exclusion criteria). Clinical diagnosis was made on the basis of clinical facts/examinations, bio-chemical tests, ultrasonography of abdomen and endoscopy. All data, collected from such patients having prior consent and ethical permissions, were analyzed.

Results: There was a male preponderance. Illiterates (39.2 %), farmers (42.5 %) and low income group of people (54.2 %) were affected more. Among the cutaneous findings, hepatic facies (39.2 %), melanosis in skin (32.5 %) and buccal mucosa (16.7 %); and, scratch marks/ pruritus and testicular atrophy (36.7 %) were more prominent as the manifestations of chronic liver disease- as our data suggested.

Conclusion: The well-known clinical signs & cutaneous manifestations of CLD remain valuable in the diagnosis of CLD which should be looked for patients with CLD, purposively.

P-0870

Hematological changes and occurrence of anemia in chronic liver disease

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Objective: The main objective of this study was to analyze hematological changes with evaluate the occurrence of anemia in patients with chronic liver disease and to detect the abnormalities of RBCs in patients with chronic liver disease and to find the type of anemia
Study Design: Descriptive type of study survey.

Setting: The study was conducted in L.U.H, hospital, from patients coming to department of Medicine OPD. Study Period: One year May, 2013 to May, 2014.

Subject and methods: The hematological changes were investigated in 100 patients with chronic liver disease, and 50 healthy control subjects. Patients were enrolled and full written consent was included in the study on the basis of diagnosis of chronic liver disease confirmed by clinical, biochemical, and ultrasonographic findings.

Results and conclusion: The hemoglobin, red blood cell, hematocrit, lymphocyte count and platelet count were significantly decreased. ESR was significantly increased in patients with chronic liver disease as compared with the healthy control subjects. The study showed 80 % had anemia which is very common in chronic liver disease. It is concluded that early diagnosis of anemia in chronic liver disease may assist a part in the treatment of patients and prevent morbidity and mortality. A Similarly in patient with chronic liver disease is correlated with infection and effect on hematological factors. However, more studies are needed to elucidate the perception of causality correlation along with severity of liver diseases and hematological factors. Keywords: Chronic liver disease, hematological changes, Anemia.

P-0871

Artificial liver support system with recombinant human growth hormone in chronic liver failure

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Objective: To observe the efficacy and safety of recombinant human growth hormone rhGH combined with artificial liver support system ALSS in the treatment of chronic liver failure.

Methods: 120 patients in Internal medicine treatment only Group A, n = 29, and in ALSS Group B, n = 43, and in ALSS combined with rhGH Group C, n = 48 in our hospital were retrospectively investigated.

Results: Total bilirubin TBIL and Prothrombin activity prothrombin time activity PTA were both improved significantly in 12 weeks in three group Reduction in TBIL A, 156.3 ± 52.0, B, 211.1 ± 63.9, C, 252 ± 39.8 umol/L, P < 0.01, Rising rates in PTA, A, 14.2 %, B, 18.5 %, C, 22.0 %, P < 0.05. There was no significant difference in TBIL and PTA in between B and C group before 2 weeks P > 0.05. However, it was significantly better in group B at 4th and 12th week than Group C P < 0.055, In the MELD, the effective rate was 25, 47.5, 70 %, in Group A, B, C P < 0.05 and it was significantly higher in Group C than B P < 0.055. The patients in group B received 125 times of treatment, the adverse reaction rate was 16.8 % 21/125, while Group C received 141 times, the adverse reaction rate was 19.14 % 27/141, P > 0.05.

Conclusion: The effect of rhGH combined with ALSS in treatment of chronic liver failure was better than that of ALSS or internal medicine only.

P-0872

Detection of blood microbiota and dysbiosis in patients with liver cirrhosis

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Background and aim: The human gut hosts a diverse community of bacteria which play an important role in liver cirrhosis (LC), and bacterial translocation explains the development of cirrhosis-related bacterial infections. We surveyed the blood microbiota by 16S rRNA gene sequencing and compared the blood microbial profiles between cirrhotic patients and control subjects.

Methods: We studied 66 patients with clinically diagnosed LC with or without hepatocellular carcinoma (HCC). Fourteen healthy individuals were also examined as controls. DNA was extracted from the whole blood samples, and the bacterial diversity and composition of each sample were determined based on 16S ribosomal RNA (rRNA) gene sequences. 16S rRNA operational taxonomic units (OTUs) were then selected.

Results: At the genus level, a total of 183 OTUs were identified in cirrhotic patients, which was considerably more diverse than in healthy controls, which showed a total of 123 OTUs. The most abundant OTU at genus level in LC patients was *Bacteroides* (21.4 %), followed by *Streptococcus* (8.5 %) and *Enterobacteriaceae* (7.8 %). With regards to comparisons between LC patients and healthy controls, the abundance of *Ruminococcus* (1.9 % v 3.5 %; p = 0.04) and *Lactobacillus* (1.1 v 1.5 %; p = 0.03) were significantly lower in LC. LC patients with HCC showed higher abundance in *Bacteroides* and *Enterobacteriaceae* and diminution in *Bifidobacterium*, while cirrhotics without HCC were not different from controls.

Conclusion: We successfully carried out the metagenomic analysis of the blood microbiome composition in patients with LC, and the results suggest the existence of dysbiosis, and moreover, underlying bacterial translocation.

P-0873

Reduced human serum albumin is a useful biomarker of progression in chronic liver disease

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Background: Human serum albumin (HSA) has micro-heterogeneity including oxidized and reduced forms. Aim of this study was to evaluate oxidative modification of albumin in chronic liver disease patients. We also evaluate the diagnostic value of reduced HSA level as a novel biomarker of classifying disease progression.

Method: 16 healthy controls and 338 patients with chronic liver disease (152 chronic hepatitis (CH) and 186 liver cirrhosis (LC)) were enrolled. We performed high performance liquid chromatography analysis of HSA and determined the proportion of reduced and oxidized HSA. Analysis of variance (ANOVA), Jonckheere-Terpstra (JT) test, receiver operating characteristic curves (ROC) were used for statistical analysis.

Results: As disease progressed, the amounts of total amounts of HSA and reduced HSA level tended to decrease ($P < 0.001$, JT test). They decreased significantly according to disease progression ($P < 0.05$), whereas the oxidized HSA/ total HSA ratios increased ($P < 0.05$) by ANOVA. By using ROC analysis, reduced HSA levels with disease progression showed the highest areas under the curves (AUC) in all parameters. The optimum cut-off value of reduced HSA levels to distinguish patients with CH from healthy controls was 3.138 g/dl (AUC 0.822; $P < 0.001$, sensitivity 68 %, specificity 100 %) and LC from CH was 2.610 g/dl (AUC 0.863; $P < 0.001$, sensitivity 80 %, specificity 87 %).

Conclusion: We could classify chronic liver disease progression with a high sensitivity and a reasonable specificity by using ROC analysis. Our findings revealed reduced HSA levels as a sensitive biomarker of chronic liver disease progression.

P-0874

Serum visfatin, HbA1c, insulin, C peptide and glucose levels in patients with liver cirrhosis

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Aim: There are important tasks of the liver in the metabolism of carbohydrates. In this study, we searched existence of insulin resistance and its relationship with the level of visfatin, glycated hemoglobin (HbA1c), insulin, C peptide and fasting blood glucose (FBG) levels in patients with liver cirrhosis.

Methods: In the study 56 liver cirrhosis patients and 30 healthy volunteer group participated. The participants are divided in to 3 groups as compensated (Group 1, n = 28) and decompensated (Group 2, n = 28) cirrhosis patients, and healthy controls (Group 3, n = 30). Visfatin, HbA1c, insulin, C peptide ve FBG levels were measured in a biochemistry autoanalyser.

Results: Age distribution between the groups was statistically different (Group 1 = 51, 60; group 2 = 61,28; group 3 = 43,10, $p = 0.001$). Sex distribution between the groups was not statistically different ($p = 0.633$). C peptide was statistically meaningfully different between the groups (Group 1 = 3,72; group 2 = 6,55; group 3 = 2,81, $p = 0.001$). FGB, insulin, Homeostasis Model Assessment (HOMA), HbA1c and visfatin levels weren't different in all groups ($p = 0.05$). Visfatin showed a positive correlation with HOMA in patients with liver cirrhosis ($r = 0.305$, $p = 0.025$).

Conclusion: On the contrary to the many previous studies, our study has showed that FBG, insulin, HbA1c, visfatin and HOMA levels of cirrhosis were similar those of the control group. The level of C peptide in cirrhosis group was significantly higher than the that in control group. Moreover there was a positive correlation between visfatin and HOMA in patients with liver cirrhosis.

P-0875

Serum transferrin isoforms in liver diseases

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The aim of this study was to assess the effect of alcoholic cirrhosis (AC), non-alcoholic cirrhosis (NAC) and toxic hepatitis (TH) on the serum profile of transferrin isoforms (Tf).

Methods: Serum samples from 107 patients (AC-58, NAC-25, TH-24) and 20 controls were collected. The samples were analyzed by capillary electrophoresis (CE) technology. The serum Tf can be separated into asialo-, di-, tri-, tetra- and pentasialotransferrin.

Results: The concentration of disialotransferrin was significantly higher in TH pts ($5.34 \pm 20.38\%$) compared to the control ($0.98 \pm 1.16\%$, $P = 0.007$). Both in AC and NAC concentrations of trisialotransferrin (6.37 ± 2.93 , $5.34 \pm 2.47\%$ respectively) were significantly higher than in the control ($3.62 \pm 1.16\%$), ($P < 0.001$, $P = 0.005$; respectively). Concentration of disialotransferrin in TH was significantly higher than that in NAC ($0.70 \pm 0.35\%$) ($P = 0.006$). Also the concentrations of tri- and tetrasialotransferrin were significantly higher in TH ($7.85 \pm 19.89\%$, $79.82 \pm 5.71\%$; respectively) than that in NAC ($5.34 \pm 2.47\%$, $77.21 \pm 3.69\%$) ($P = 0.001$, $P < 0.001$; respectively). There were significant differences between tri-, tetra- and pentasialotransferrin according to Child-Pugh score in cirrhotic patients ($P = 0.003$, $P < 0.001$, $P < 0.001$; respectively). Post-hoc analysis revealed that tri- and pentasialotransferrin were significantly higher in C score (7.22 ± 3.11 , $23.08 \pm 15.90\%$; respectively) than that in A score (4.59 ± 1.85 , $15.68 \pm 2.33\%$; respectively) ($P = 0.001$, $P = 0.001$; respectively), and pentasialotransferrin was higher in C score than that in B score ($16.62 \pm 4.44\%$) ($P = 0.005$). The tetrasialotransferrin isoforms were higher in A score ($78.99 \pm 3.00\%$) and B score ($76.65 \pm 3.59\%$) than that in C score ($69.05 \pm 14.25\%$) ($P < 0.001$).

Conclusions: The concentrations of Tf isoforms are higher in toxic hepatitis than that in cirrhosis, and differ according to the severity of liver cirrhosis.

P-0876

Effect of vitamin B6, vitamin B12 and folic acid on serum homocysteine level

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Background: Hyperhomocysteinemia is known risk factor of cardio-cerebrovascular Disease. We studied to evaluate the effect of vitamin B₆, vitamin B₁₂, and folic acid treatment on serum homocysteine level.

Methods: In this retrospective cohort study, we reviewed 945 visitors' medical records who were measured serum homocysteine level

and prescribed vitamin B complex. 122 suitable subjects were divided into two groups of vitamin B complex once daily and vitamin B complex plus folic acid once daily. We analyzed changes of serum homocysteine level in each group before and after taking vitamin B complex and folic acid.

Results: The mean follow up period was 26 weeks and mean treatment period was 18 weeks. Serum homocysteine level decreased 22.1 % ($p < 0.001$) in vitamin B complex group ($n = 92$) and 23.5 % ($p < 0.001$) in vitamin B plus folic acid group ($n = 30$). In two group comparison, the baseline and follow up homocysteine level showed no difference. The variation also showed no difference between two groups ($p = 0.578$) which means adding folic acid on vitamin B complex has no additional effect. Among 21 smokers, 8 stopped smoking and 22 problem drinkers, 9 cut down drinking at follow up point. By analyzing before and after serum homocysteine level, we found smoking cessation ($p = 0.587$) and moderation in drinking ($p = 0.367$) both have no relation to serum homocysteine level.

Conclusion: Vitamin B₆ and vitamin B₁₂ lowers serum homocysteine level. Adding folic acid do not have an additional beneficial effect on serum homocysteine level lowering to who taking vitamin B₆ and vitamin B₁₂.

P-0877

Vitamin D deficiency in chronic liver disease patients of the Philippine General Hospital

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Background: Low vitamin D levels result in higher incidence of liver fibrosis and cirrhosis, poor treatment response, and increased morbidity and mortality in patients with chronic liver disease (CLD). This study assessed whether CLD patients in the Philippines, despite adequate sunlight exposure, have vitamin D deficiency and whether this is associated with poor outcomes.

Methodology: Consecutive CLD patients at the outpatient clinics of PGH were included. Clinical data such as age, gender, BMI, etiology of CLD, presence of cirrhosis and ascites, and daily sun exposure were recorded. Standard biochemical liver tests within 3 months of enrolment into the study, such as alanine and aspartate aminotransferases, prothrombin time, total bilirubin, and albumin were documented. Child Pugh scores for cirrhotic patients were computed. Serum vitamin D was determined using the ARCHITECT chemiluminescent microparticle assay. Univariate analysis and simple logistic regression were used to determine independent predictors of vitamin D deficiency. A p-value of less than 0.05 was considered as statistically significant.

Results: A total of 72 patients were included. The prevalence of vitamin D deficiency (less than 20 ng/mL) was 6.9 %; insufficiency (20.1 to 29 ng/mL) 52.8 %; and sufficiency (greater than 30 ng/mL) 40.3 %. Both univariate analysis and logistic regression showed no statistical difference among vitamin D deficient, insufficient, and sufficient subjects in terms of etiology and factors affecting the severity of CLD.

P-0878

Vitamin D levels in cirrhosis and chronic liver disease

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Objective: The objective of this study was to assess the level of Vitamin D in cirrhosis and chronic liver disease.

Method: Study was carried out at Liaquat University Civil Hospital Hyderabad and Jamshoro from April 2014 to December 2014. Vitamin D level was analyzed in 50 controls and 100 patients with cirrhosis and chronic liver disease, age group ranged from 21 to 65 years. Vitamin D level was determined on automated instrument Architect i1000SR ABBOTT kit method were used and the reagent kit was obtained from Abbott. Data was analyzed using SPSS: 16.

Results: Low vitamin D levels were detected in patients as compared to the control subjects and very high frequency of vitamin D deficiency in cirrhosis and CLD patients.

Conclusion: This study highlighted vitamin D is significantly deficient in cirrhosis and chronic liver disease. Further investigations should be required for understanding of vitamin D metabolism and vitamin D deficiency in patients with cirrhosis and chronic liver disease.

Keywords: Vitamin D, LUMHS, Students, Employees, Health.

P-0879

Prevalence of vitamin d deficiency in patients with chronic viral hepatitis: a Nepalese study

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Background: Recent studies have shown that vitamin D deficiency is common in patients with chronic viral hepatitis and this may have some impact on treatment of these diseases. There is no study on prevalence of Vitamin D deficiency in patients of chronic viral hepatitis from Nepal.

Aim: To determine the prevalence of vitamin D deficiency in Nepalese patients with chronic viral hepatitis B and C.

Methods: A Serum level of 25 OHD was estimated in consecutive chronic viral hepatitis B and C patients attending outpatient department of liver unit, Bir hospital. Patients who were taking vitamin D supplement were excluded from the study.

Results: Among 400 patients enrolled, 320 (80 %) had chronic hepatitis B and 80 (20 %) had chronic hepatitis C. 240 (60 %) patients were male and 160 (40 %) were female. Overall, 150 (37.5 %) had vitamin D deficiency (less than 20 ng/ml), 200 (50 %) had vitamin D insufficiency (20-30 ng/ml), and 50 (12.5 %) were vitamin D sufficient (more than 30 ng/ml). Among chronic hepatitis C patients, 35 (43.75 %) had vitamin D deficiency and 40 (50 %) had vitamin D insufficiency, 5 (6.25 %) were vitamin D sufficient. Among chronic hepatitis B patients, 130 (40.6 %) had vitamin D

deficiency and 145 (45.3 %) had vitamin D insufficiency, 45 (14.1 %) were vitamin D sufficient.

Conclusion: We found that most of the patients with chronic viral hepatitis in Nepal have vitamin D deficiency and insufficiency. Further larger studies are needed to assess this issue and to determine its impact on treatment outcomes of these diseases.

P-0880

Copper deficiency in liver disorders: implications for disease mechanism and clinical management

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Copper is an essential cofactor in energy metabolism, antioxidant defense and the innate immune system. The association between copper deficiency and liver disease has not been firmly established. The aim of the study is to describe 10 patients who presented with liver disorders and copper deficiency, defined by low serum (<80 µg/dL, N = 9), hepatic (<10 µg/g dry weight, N = 5) copper or both (N = 4). Serum copper was measured during assessment for Wilson’s disease or malnutrition. Hepatic copper was measured on biopsy or explant. The median age was 36. Five were male. Table 1 provides clinical data and Figure 1 plots serum and hepatic copper concentrations. Pathology showed cirrhosis in 8 and steatohepatitis in 6. Malnutrition was present in 6. Known associations with copper deficiency were present in 4: gastric bypass (2) and Crohn’s (2). Both subjects with gastric bypass presented with acute-on-chronic liver failure (ACLF). One recovered following parenteral nutrition including copper. The other died following oral copper and nutritional supplementation. One case with inactive Crohn’s who presented with ACLF made full recovery after 6 months of oral copper (2 mg copper gluconate daily) with normal synthetic function and disappearance of ascites

Conclusion: Copper deficiency occurs in a range of liver diseases. It correlated with low hepatic copper in a subset, suggestive of systemic deficiency. When malnutrition was present, copper supplementation reversed features of hepatic decompensation. Given the physiological importance of copper, its deficiency may represent a novel mechanism of liver injury as well as a potential target of therapy

Table 1. Summary of cases who presented with copper deficiency and liver diseases

Case number	Age	Sex	Liver disease presentation	Clinical features suggestive of copper deficiency	Other medical history	Serum Cu (µg/dL)	Hepatic Cu (µg/g)	Urine Cu (µg)	Liver histology	Clinical outcome
1	18	Male	Acute liver failure of unknown etiology	None	None	71	<10	157	Necrosis, minimal fibrosis and iron	Transplantation, alive
2	27	Male	Decompensated cirrhosis, HCV	Anemia	Factor VIII deficiency	19	26	NA	Cirrhosis, minimal iron	Died
3	31	Male	Abnormal liver enzymes, NAFLD	None	Perianal Crohn’s	50	38	9	Steatosis, minimal fibrosis and iron	Asymptomatic, alive
4	34	Female	Acute-on-chronic liver failure, NASH	Malnutrition	Obesity, gastric-bypass	41	NA	45	Steatohepatitis, bridging fibrosis	Treated with parenteral nutrition, full recovery of liver function
5	35	Female	Decompensated cirrhosis, ALD	Malnutrition, unexplained anemia and iron overload	None	NA	<10	NA	Cirrhosis, no steatosis, moderate iron	Died
6	37	Female	Decompensated cirrhosis, ALD	Anemia, unexplained anemia and iron overload	None	63	17	NA	Cirrhosis, no steatosis, moderate iron	Listed for transplantation, alive
7	41	Male	Decompensated cirrhosis, NASH	Malnutrition	Obesity	37	<10	NA	Cirrhosis, steatohepatitis, minimal iron	Transplantation, alive
8	44	Female	Acute-on-chronic liver failure, NASH	Malnutrition, unexplained anemia, recurrent infections	Obesity, gastric-bypass	36	<10	NA	Steatohepatitis, bridging fibrosis, minimal iron	Died from multiorgan failure
9	58	Female	Decompensated cirrhosis, NASH	Malnutrition, anemia	Crohn’s, ileum and right colon resection	41	<10	NA	Cirrhosis, minimal iron	Treated with oral nutrition, copper supplement, full recovery of liver function
10	64	Male	Decompensated cirrhosis, HBV and HCC	Anemia, recurrent infections, unexplained iron overload	None	42	77	NA	Cirrhosis with severe iron overload	Transplantation, recurrent HCC within 6 months

*Serum copper (Cu) normal range: 80-155 µg/dL; †Hepatic copper normal range: 10-35 µg/g dry weight; ‡Urinary copper: 15-80 µg/L

P-0881

Zinc and copper concentration in children with chronic liver disease

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Background and objective: Chronic liver disease, particularly the one related to viral hepatitis B and C, is responsible for a significant disease burden and cause of mortality worldwide. The present study was designed to determine the zinc and copper concentration in children with chronic liver disease as compared with the healthy controls.

Material and method: A total of hundred children with and without chronic liver disease were selected. Blood samples were made to clot before serum was separated. Determination of Copper and zinc concentration were analyzed using atomic absorption spectrophotometry.

Results and conclusion: Serum copper was significantly increased, while serum zinc concentration was significantly decreased in the children with chronic liver disease as compared with the healthy controls. Serum Copper was associated positively with biochemical parameters of liver damage; however serum Zinc was correlated negatively with biochemical parameters of liver damage. Serum Zinc and Copper as biomarkers for observing the severity of liver damage in children with chronic liver disease.

Keywords: chronic liver disease, copper, zinc, children

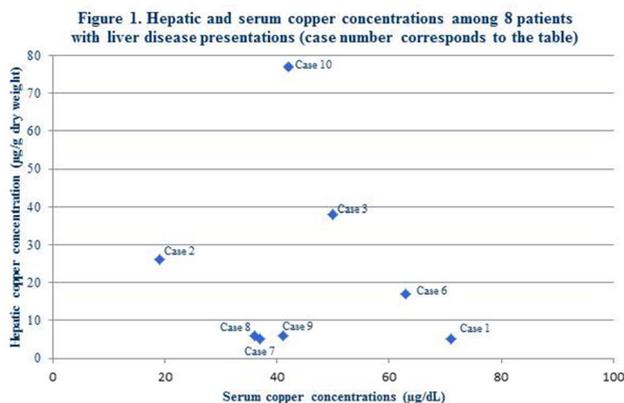


Figure 1. Hepatic and serum copper concentrations among 8 patients with liver disease presentations (case number corresponds to the table)

P-0882

The correlation between serum zinc level and dysgeusia in cirrhotic patients

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Introduction: Cirrhosis is associated with several complications, such as hypo-albuminemia, poor appetite and dysgeusia. Since serum zinc levels have been reported to contribute to dysgeusia, the aim of the study is to identify the correlation between serum zinc levels and dysgeusia in cirrhotic patient.

Method: This is a cross sectional study. The study duration was from July, 2014 to September, 2014. 26 patients with cirrhosis were enrolled into the study. The disease severity is from Child-Pugh score A to C. Exclusion criteria are patients with conscious unclear, and those who were receiving parental nutrition. The blood examinations included serum zinc, and albumin levels.

Results: Among 26 patients, 7 were Child-Pugh A, 7 were Child-Pugh B, and 12 were Child-Pugh C. Serum zinc levels were from 35 to 105 $\mu\text{g}/\text{dl}$. The data showed that zinc levels and dysgeusia were positive relation (no dysgeusia 66.00 ± 21.25 , dysgeusia $48.67.83 \pm 14.73$, $p < 0.05$). Moreover, the severity of liver function was significantly correlated with serum zinc levels ($p < 0.05$), and the serum zinc levels were significantly higher in Child-Pugh A than C ($p < 0.05$).

Conclusion: Serum zinc levels were correlated with dysgeusia in cirrhotic patients. Taste change happened when zinc deficiency was noted in cirrhotic patients.

Keywords: Cirrhosis, Zinc deficiency, dysgeusia

P-0883

Impact of acute Fe and Cu administration on hematology and biochemistry in *Rattus norvegicus*

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Introduction: Essential trace elements; iron (Fe) and copper (Cu) are entailed in a wide range of fundamental biological reactions, crucial for cellular functions, as their necessity resides in ability to contribute in one-electron exchange reactions. However, the same attributes pertinent to their essentiality also impart role in potentially detrimental conditions due to their surplus amount in body. The current study's aim was to elucidate toxic effects of Fe&Cu on hematology and biochemistry in rat.

Methods: Adult male Wistar Rats (200 g) were used and three experimental groups (G-I: FeSO_4 , 60 mg/kg; G-II: CuSO_4 , 10 mg/kg; G-III: FeSO_4 , 30 mg/kg and CuSO_4 , 5 mg/kg) were established against a control group. Animals were sacrificed 24 h of acute intraperitoneal administration of Fe and Cu. Blood samples were collected and processed for hematological and biochemical tests.

Results: Hematological and biochemical analysis revealed statistically significant marked variations in RBC ($P = 0.0024$), MCV ($P = 0.0003$), HCT % ($P = 0.0009$), Hb ($P = 0.0076$), MCH ($P = 0.05$), thrombocytes ($P = 0.0009$), lymphocytes %

($P = 0.0305$), cholesterol ($P = 0.0002$), triglycerides ($P < 0.0001$), serum urea ($P = 0.0072$), uric acid ($P = 0.0089$) and chloride ($P = 0.0413$), in experimental groups as compared to control group, when analyzed by one-way ANOVA with post-hoc Tukey's test. On the other hand, a significant decline was observed in RDW % ($P = 0.0004$), MCHC ($P = 0.0018$), MPV ($P < 0.0001$) and neutrophils % ($P = 0.0071$), HDL-cholesterol ($P = 0.0094$) and LDL-cholesterol ($P = 0.0046$) and potassium ($P = 0.0166$). However, statistically non-significant variations were observed in WBCs, monocytes %, eosinophils %, creatinine ($P = 0.2560$), sodium ($P = 0.9057$), calcium ($P = 0.3309$) and phosphate ($P = 0.7776$) in experimental groups.

Conclusion: It is deduced that Fe and Cu intoxication in rats, manifested remarkable impact on hematology, lipid profile, renal function tests and serum electrolytes; indicating an aspect that despite of being important, Fe and Cu also execute deleterious effects due to their excess in body.

Table: Results indicate mean value \pm S.E.M. (Level of Significance $P < 0.05^*$, 0.01^{**} , 0.001^{***} analyzed by one-way ANOVA in combination with post-hoc Tukey test).

HEMATOLOGY:					
Group	RBCs ($\times 10^{12}/\text{l}$)	MCV (fl)	RDW (%)	HCT (%)	
Control	4.77 \pm 0.24	40.65 \pm 0.15	14.50 \pm 0.10	30.37 \pm 0.27	
I	9.01 \pm 0.20	46.23 \pm 0.12	13.90 \pm 0.23	39.53 \pm 0.77	***
II	8.54 \pm 0.30	46.23 \pm 0.72	13.90 \pm 0.23	39.53 \pm 0.77	***
III	8.91 \pm 0.10	44.30 \pm 0.40	14.50 \pm 0.11	39.50 \pm 0.11	***
Platelets ($\times 10^9/\text{l}$)					
Group	Significance	MPV (fl)	Hemoglobin (g/dl)	MC (Hgb)	
Control	457.00 \pm 14.04	7.40 \pm 0.05	12.83 \pm 0.92	17.00 \pm 0.10	
I	709.00 \pm 16.00	6.50 \pm 0.08	16.14 \pm 0.23	17.93 \pm 0.33	***
II	729.00 \pm 20.00	6.80 \pm 0.05	16.47 \pm 0.63	18.95 \pm 0.45	***
III	735.30 \pm 29.50	6.70 \pm 0.05	15.70 \pm 0.11	17.77 \pm 0.14	***
Lymphocytes (%)					
Group	Significance	Neutrophils (%)			
Control	42.72 \pm 0.95	45.50 \pm 0.50	51.50 \pm 0.50		
I	38.47 \pm 0.64	52.00 \pm 0.09	44.50 \pm 0.50		
II	40.03 \pm 0.63	52.00 \pm 0.57	41.33 \pm 0.33		***
III	40.10 \pm 0.55	51.50 \pm 0.50	44.71 \pm 0.20		***

BIOCHEMISTRY:					
Group	Cholesterol (mg/dl)	Triglycerides (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	
Control	78.67 \pm 7.68	63.00 \pm 7.23	61.00 \pm 8.00	118.03 \pm 0.00	
I	116.24 \pm 0.50	111.70 \pm 0.60	27.90 \pm 0.05	91.07 \pm 0.18	***
II	149.34 \pm 3.38	124.74 \pm 3.28	40.00 \pm 1.15	94.33 \pm 3.84	***
III	140.74 \pm 3.28	113.74 \pm 0.91	36.33 \pm 0.88	96.07 \pm 3.28	***
Urea (mg/dl)					
Group	Significance	Uric acid (mg/dl)	Potassium (mmol/L)	Chloride (mmol/L)	
Control	31.50 \pm 0.50	5.30 \pm 0.23	5.15 \pm 0.73	96.00 \pm 0.00	
I	31.67 \pm 1.76	6.60 \pm 0.20	3.40 \pm 0.15	100.51 \pm 0.50	
II	29.33 \pm 0.88	5.30 \pm 0.25	4.40 \pm 0.23	99.67 \pm 1.45	***
III	32.50 \pm 1.50	5.70 \pm 0.17	4.93 \pm 0.12	100.54 \pm 0.50	***

(Control, I: FeSO_4 treatment (60mg/kg); II: CuSO_4 treatment (10mg/kg); III: FeSO_4 (30mg/kg) & CuSO_4 (5mg/kg) treatment for 24 hours).

*: significance level when compared to control. **: significance level when compared to Group I. c: significance level when compared to Group II.

P-0884

CD36 deficiency aggravates the development of NASH via up-regulating MCP-1 expression in hepatocytes

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Cluster of differentiation 36 (CD36), a member of the class B scavenger receptor family (also called fatty acid translocase, FAT), is involved in the development of non-alcoholic steatohepatitis (NASH) by unknown mechanisms. In this study, we found CD36 deficiency in cultured hepatocytes and CD36 gene knock-out mice ($\text{CD36}^{-/-}$), increased monocyte chemoattractant protein-1 (MCP-1) expression of hepatocytes and induced macrophage infiltration, finally promoting hepatic inflammatory response and fibrosis. The degree of inflammation and fibrosis could be alleviated after inhibition of macrophage migration by gadolinium chloride. Acetyl Histone3 (H3), which

usually inhibited by histone deacetylases (HDACs), is a transcriptional factor which binds to MCP-1 promoter. We demonstrated that the nuclear expression of HDAC2 which highly expresses in wild type hepatocytes was reduced in CD36 deficiency hepatocytes. As a consequence, the level of acetyl Histone3 (H3) binding to MCP-1 promoters was increased in CD36 deficient hepatocytes, causing hepatic specific MCP-1 transcriptional activation. Moreover, reduction of activity of HDACs in both CD36^{-/-} mice liver and cultured hepatocytes was due to reduction of intracellular ROS level, while supplement of low concentration H₂O₂ was able to recover the nuclear expression of HDAC2, therefore attenuated the level of acetyl H3 binding to MCP-1 promoters and decreased MCP-1 gene expression.

Conclusion: CD36 deficiency would promote the development of NASH via the elevation of hepatocytes MCP-1 expression and the subsequently increased macrophage infiltration in liver. Targeting the reduced ROS and the suppressed nuclear HDAC2 in hepatocytes might be an effective strategy for the prevention of NASH in CD36 deficient population.

P-0885

Cyclodextrin promotes liver inflammation and fibrosis in high fat/cholesterol/choleate diet fed mice

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Background: 2-hydroxypropyl beta cyclodextrin (HPBCD) is often used as a vehicle for oral drug administration but also alters cholesterol absorption and metabolism. Here we investigated whether HPBCD administration affects liver complications in mice fed a 60 % high fat, 1.25 % cholesterol, 0.5 % cholic acid diet for 3 weeks.

Methods: C57BL6/J mice were fed a 60 % high fat, 1.25 % cholesterol, 0.5 % cholic acid diet for 3 weeks. This 3-week diet was initially found to increase lipids levels and expression of genes involved in inflammation and fibrosis in the liver, as compared with chow fed mice. After 1 week of diet, mice were treated for 2 weeks with drinking water (control) or 20 % HPBCD by oral gavage once daily.

Results: Compared to control mice, mice treated with HPBCD for 2 weeks showed 20 % higher liver weight ($p < 0.001$). Plasma ALT and AST levels were 56 and 45 % higher with HPBCD, although not significantly. Hepatic total cholesterol, triglycerides and fatty acids levels were increased by 118, 46 and 94 % with HPBCD (all $p < 0.001$ vs. control). Histology analysis and NAS scoring indicated that control mice showed no inflammation (score 0.4/3) and fibrosis (score 0.1/3). In contrast, HPBCD treated mice showed a strong induction of inflammation (score 2/3) and fibrosis (score 1/3).

Conclusion: The present study indicates that HPBCD oral administration induces inflammation and fibrosis in mice fed a 60 % high fat, 1.25 % cholesterol, 0.5 % cholic acid diet in 3 weeks. This model seems relevant to rapidly induces liver complications and evaluate drugs targeting non-alcoholic steatohepatitis.

P-0886

Distribution of hepatic stellate cells which express LRAT and/or CRBP-1 in NASH patients

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Background: Precisely what type of cells mainly contribute to cent-lobular fibrosis with NASH still remains unclear. Many reports suggest that hepatic stellate cells (HSCs) in the parenchyma are activated and transformed fibrogenic myofibroblast in the liver with NASH. It is necessary to clarify the characteristics of cells that contribute to cent-lobular fibrosis in order to determine the mechanism of fibrogenesis to develop a therapeutic target for fibrosis and carcinogenesis on NASH.

Aims: The present study was undertaken to examine whether LRAT+ and/or CRBP-1+ HSCs contribute to cent-lobular fibrosis on NASH.

Methods: Antibodies to lecithin:retinol acyltransferase (LRAT), cellular retinol binding protein-1 (CRBP-1) and a widely ascertained antibody to activated HSCs (α -smooth muscle actin; α -SMA), and anti R58 monoclonal antibody to TGF- β latency associated protein (LAP-D) in cells or matrix were used for immunohistochemical studies to assess the distribution of cells that contribute to the development of fibrosis.

Results: The LRAT+ and/or CRBP-1+ HSCs were stained in cent-lobular lesion in fibrotic liver with NASH. These cells were observed in fibrotic septa, which was stained with α -SMA and/or LAP-D.

Conclusion: The present study provides evidence that functional HSCs expressing LRAT and/or CRBP-1 that continue to maintain the ability to store vitamin A contribute in the development of cent-lobular fibrosis in addition to parenchymal fibrosis in patients with NASH.

P-0887

Duodenal jejunal bypass improves non-alcoholic steatohepatitis in rats

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Background: It has been reported that the bariatric surgery improves metabolic comorbidities such as diabetes mellitus and non-alcoholic

steatohepatitis (NASH). Bile acids (BA) and its nuclear receptor, farnesoid X receptor (FXR), are thought to be involved in this mechanism. The present study investigated both effects of bariatric surgery on NASH improvement and its mechanisms by using a novel NASH model rat.

Methods: Male Sprague-Dawley rats fed by a high fat and high fructose diet, which develop obesity, insulin resistance and NASH were used. On this NASH rats, we performed duodenal jejunal bypass (DJB), one of the bariatric procedures.

Results: DJB suppressed weight gain after surgery, improved insulin resistance and ameliorated NASH especially by suppressing the inflammatory reaction. In rats received DJB, the plasma BA level was elevated, and hepatic mRNA expression of FXR and its target transcription factor, small heterodimer partner (SHP), were increased as well. The FXR/SHP signal is known to have an anti-inflammation effect, which may account for observed improvement of NASH. Besides, the plasma BA level in portal vein was also elevated after DJB, and expression of hepatic BA transporters that carries BA to systemic circulation were increased as well. Thus, increase of both BA reabsorption and efflux to systemic circulation seem to be a key mechanism of elevation of the systemic plasma BA level after DJB.

Conclusion: DJB improves NASH, and increased plasma BA may play a major role, especially in terms of an anti-inflammation effect.

P-0888

Dysregulation of hepatic 27-hydroxycholesterol in steatohepatitis model mice with hyperglycemia

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Background: Twenty seven-hydroxycholesterol (27HC), one of the major oxysterol (OS) found in the human circulation, is produced by the mitochondrial enzyme CYP27A1. Previous studies demonstrated that hepatic inflammation was increased by genetic deletion of CYP27A1 and decreased by 27HC treatment in high fat diet (HFD) fed steatotic mice, however, direct cause of dysregulation of CYP27A1 and 27HC in steatohepatitis is unknown. AIM: The current study was undertaken to examine the change of hepatic OS levels in the Stelic Animal Model (STAM) mice, a validated and widely used as NASH model.

Methods: In STAM mice, 200 µg of streptozotocin was injected at the birth period, and then feeding of HFD was given from 4 weeks after injection. Extracted sterols in the liver tissue homogenates were determined by LC-MS/MS.

Results: (1) Concentration of hepatic cholesterol (CHOL): Hepatic CHOL concentrations were significantly elevated at 20 weeks in HFD fed mice (4.7 µg/mg ww) and STAM mice (4.2 µg/mg ww) compared to CTL (2.2 µg/mg ww). (2) Concentration of hepatic 27HC: 27HC was significantly lower in STAM mice compared to either of CTL or HFD mice over time. In addition, hepatic CYP27A expression level was also suppressed in STAM mice, but not in HFD mice.

Conclusion: Reduced hepatic 27HC concentration as well as CYP27A1 expression level observed in this model, is supportive the hypothesis proposed by the previous reports. Enhancement of the reduction of hepatic 27HC concentration in this model suggested a linkage between abnormality in glucose metabolism and dysregulation of CYP27A1.

P-0889

Extracellular vesicles: exploring a novel biomarker in nonalcoholic fatty liver disease pathogenesis

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Nonalcoholic fatty liver disease (NAFLD) is currently the most prevalent liver disease, however there is still high demand for a diagnostic tool that is reliable and non-invasive. This study explored whether extracellular vesicles (EVs) released from metabolically stressed tissues are useful biomarker candidates in NAFLD. C57Bl/6 mice were fed a high-fat diet (HFD; 45 %cal fat) or chow for 12 and 50 weeks. At termination, EVs were isolated from blood plasma by ultracentrifugation. Livers were diced and cultured in particle-free media from which EVs were similarly isolated. Additionally, EVs were taken from media conditioned by hepatocyte cell lines grown in low (5.5mM) or high (25 mM) glucose and supplemented with fatty acid (palmitate; 50-150 µM) or a vehicle control. EVs were characterised by electron microscopy and quantified by NanoSight. At 50 weeks, HFD mice were heavier than chow mice (60.4 ± 1.6 vs. 30.8 ± 1.2g) and had proportionally larger livers (9.2 ± 1.0 vs. 4.2 ± 0.6 % body mass). Comparable but less pronounced trends were seen at 12 weeks. Circulating EVs were increased in HFD compared to chow, while liver-derived EVs per gram tissue decreased in a time-dependent manner. At the cellular level, hepatocyte EVs increased in high glucose conditions, while fatty acid had relatively little effect on their release. The size of EVs remained stable across all studies, peaking at 100–200 nm. This was consistent with electron microscopic observations. Our study has described a model of NAFLD progression in which EVs are a potential biomarker of disease severity, and has examined the possible stimuli for their release.

P-0890

Fibroblast growth factor 21 concentration in obese children with nonalcoholic fatty liver disease

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Aim: Fibroblast growth factor 21 (FGF21) is a peptide hormone that was found to increase in patients with metabolic syndrome and NAFLD. However, its role as the biomarker of intrahepatic lipid content is not clear. Therefore the aim of the study was to evaluate the serum concentration of FGF21 in obese children.

Methods: The prospective study included 102 obese children admitted to our Department to diagnose initially suspected liver disease. Patients with viral hepatitis (HCV, HBV, CMV), autoimmune (AIH), toxic and metabolic (Wilson's disease, alpha-1-antitrypsin deficiency) liver diseases were excluded. NAFLD was diagnosed in children with liver steatosis in ultrasound as well as elevated ALT

serum activity. The degree of liver steatosis in ultrasound was graded according to Saverymuttu scale. Advanced steatosis was defined as a score > 2. The total intrahepatic lipid content (TILC) was assessed by magnetic resonance proton spectroscopy (1H-MRS).

Results: Significant positive correlation was found between FGF21 and aspartate transaminase, gamma-glutamyl transferase, total cholesterol, triglycerides, glucose, insulin, HOMA-IR, steatosis grade and TILC. FGF21 level was significantly higher in children with NAFLD (n = 36) compared to the control group (n = 24). Median FGF21 was significantly higher in NAFLD children compared to other obese patients (n = 66). Children with advanced liver steatosis had significantly higher level of FGF21 than patients with mild steatosis in ultrasound. The ability of serum FGF21 to diagnose advanced liver steatosis was significant (AUC = 0.7992, p < 0.001, sensitivity = 100 %, specificity = 72 %, cut-off >208.35 pg/ml).

Conclusion: FGF21 can be regarded as a suitable biomarker in predicting advanced liver steatosis in obese children.

P-0891

Effect of polydatin to ApoE^{-/-} mice the expression of MDA/T-SOD and liver function

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Objective: To study the effect of polydatin (PD) on atherosclerosis (AS) mice and observe the change of total-superoxide dismutase (T-SOD), malonaldehyde (MDA) and liver function.

Method: The defects of ApoE^{-/-} mice were fed with high fat diet to establish atherosclerosis mice model. ApoE^{-/-} mice were divided into 4 groups (n = 10): model group, low and high dose group of PD, simvastatin group, besides, we set C57BL/6J mice as normal group. After 12 weeks treated with different drugs, T-SOD, MDA in the liver tissue, the blood glucose and the blood lipid and the liver function were detected. And then to observe the change of liver tissue pathology morphology that was stained with HE.

Results: Compared with normal group, blood glucose, and the levels of TC, TG, LDL-C, ALT, AST, and MDA in the liver tissues in model group had increased (P<0.01) and SOD in liver tissues and the plasma HDL-C had significantly decreased (P<0.01). Compared with the model group, polydatin treated mice had show the significant changes in blood glucose, ALT, AST, TC, TG, LDL-C, HDL-C and MDA, SOD in liver tissue and liver morphology (P<0.05).

Conclusion: Polydatin can relieve the liver injury by down-regulating the expression of MDA and up-regulating SOD in liver tissue, and can relieve the injury of atherosclerosis by down-regulating TC, TG, LDL-C and up-regulating HDL-C, which may be the mechanism of anti-atherosclerosis.

Keywords: Polydatin; Atherosclerosis; Liver function; Oxidative stress

P-0892

Ipragliflozin ameliorates liver fibrosis development in diabetic OLETF rats

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It is widely understood that insulin resistance (IR) critically correlates with liver fibrosis development in chronic liver injuries. Several reports have proved that anti-IR treatment can alleviate liver fibrosis. A sodium glucose cotransporter2 inhibitor (SGLT2-I) is a new anti-diabetic agent that inhibits glucose reabsorption in renal proximal tubules. This study was aimed to elucidate the effect of an SGLT2-I on the development of liver fibrosis using diabetic Otsuka Long-Evans Tokushima Fatty (OLETF) rats and non-diabetic Long-Evans Tokushima Otsuka (LETO) rats. Rats were intraperitoneally injected with porcine serum twice a week for 12 weeks to augment liver fibrogenesis. Different concentrations of ipragliflozin (3 and 6 mg/kg) were orally administered during the experimental period. Serological and histological data were examined at the end of experimental period. The direct effect of ipragliflozin on the proliferation of a human hepatic stellate cell (HSC) line, LX-2, was also evaluated in vitro. OLETF rats, but not LETO rats, exerted severe fibrosis by porcine serum injection. Treatment with ipragliflozin markedly attenuated liver fibrosis development and expression of hepatic fibrosis markers along with improvement of IR in a dose dependent manner in OLETF rats but not in LETO rats. In contrast, proliferation of LX-2 was not affected by ipragliflozin treatment suggesting ipragliflozin had no direct effect on proliferation of HSCs. In conclusion, our dataset suggest that an SGLT2-I could alleviate liver fibrosis development by improving IR. This may be one of new therapeutic strategy for chronic liver diseases with IR.

P-0893

Lipid-induced endoplasmic reticulum stress impairs selective autophagy in hepatocytes

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Aim: Several studies have showed that blockage of hepatic autophagy occurred in nonalcoholic fatty liver disease. However, the mechanism of this blockage remains unclear. In this study, we evaluated how lipid overload is linked to impairment of autophagy in hepatocytes.

Methods and results: Saturated fatty acids (SFAs) but not monounsaturated fatty acids (MUFAs) induced endoplasmic reticulum (ER) stress and accumulation of autophagosomes. Microtubule-associated protein 1 light chain 3 (LC3) turnover assay demonstrated that SFAs induced the reduction of lysosomal degradation of

contents of autophagosomes. Moreover, we monitored autophagic flux in cells transfected with mRFP-GFP tandem fluorescence-tagged LC3. GFP-fluorescence is quenched in the lysosome but mRFP-fluorescence is not. A significant increase in mRFP and GFP overlapping was found in cells treated with SFAs compared with MUFAs. SFAs also suppressed co-localization of mRFP and lysosome-associated membrane protein 1 (Lamp1), suggesting that SFAs-induced impairment of autophagic flux was due to blockage of autophagosome-lysosome fusion. We also found that this impairment occurred in an ER stress-dependent manner. Moreover, immunofluorescence analysis confirmed that ubiquitin and p62-positive inclusions were observed in SFAs-treated cells. And Ser351-phosphorylated p62, which is indispensable for selective autophagy of aggregated proteins, increased only in SFAs-treated cells. In addition, SFAs-induced accumulation of Ser351-phosphorylated p62 and ubiquitinated proteins further increased upon administration of bafilomycin A1, a lysosomal proteinase inhibitor (vacuolar- H+ATPase inhibitor). This phenomenon implies that selective autophagic sequestration of aggregated proteins is not inhibited in SFAs-treated cells.

Conclusion: Lipid-induced ER stress impairs selective autophagy at the step of autophagosome-lysosome fusion in hepatocytes.

P-0894

Ether phospholipid profile in mice with non-alcoholic steatohepatitis

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Aim: The plasma levels of plasmalogen, a class of ether phospholipids, are known to be decreased in patients with non-alcoholic steatohepatitis (NASH). However, there is still less information on ether phospholipids composition at species level. This study was designed to evaluate ether phospholipid composition in both plasma and liver of a fibrotic NASH mouse model and to assess the association between ether phospholipids and NASH.

Method: C57BL/6J male mice were fed a choline deficient, L-amino acid-defined high-fat diet (CDAHFD) or a control chow for 13 weeks ($n = 6$ per group). The relative amounts of ether phospholipids in plasma and liver were measured using liquid chromatography-mass spectrometry.

Result: Mice fed a CDAHFD for 13 weeks exhibited steatosis, inflammation and fibrosis in the liver. In these mice compared with controls, plasma levels for phosphatidylethanolamine (PE)-based ether lipids, specifically PE (O-16:0/18:1), PE (O-18:1/20:3), PE(O-38:4), PE (O-40:4), PE (P-16:0/18:1), PE (P-16:0/20:4), PE (P-16:0/22:6), PE (P-40:5) and PE (P-40:6) were significantly lower ($P < 0.05$ for all) whereas hepatic levels for these PEs were higher. Significant higher levels of polyunsaturated fatty acid (PUFA)-containing ether phosphatidylcholines (PC), specifically PC (O-18:1/20:3), PC (O-18:0/20:4), PC (P-18:0/20:4) and PC (O-34:1), were observed in both plasma and liver ($P < 0.05$ for all).

Conclusion: The levels for specific ether phospholipid subspecies were altered in plasma and liver of mice with fibrotic NASH, suggesting its potential role in disease progression or as a biomarker.

P-0895

Rapid progression of hepatocellular carcinoma in Neuropeptide Y knockout mice under high fat diet

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Background: Nonalcoholic fatty liver disease (NAFLD) is currently recognized as the possible cause of hepatocellular carcinoma (HCC), and neuropeptide Y (Npy) is well documented to regulate the energy metabolism in mammals.

Aim: The aim of this study is to analyze the impact of Npy on the carcinogenesis and progression of hepatocellular carcinoma (HCC), using the model of Npy-knockout mice under high fat diet (HFD).

Materials and methods: Npy-knockout mice (Npy^{-/-}) were divided into 3 groups, calorie-restricted group (CR), fed with ad libitum of regular diet group (RD), and fed with ad libitum of high fat diet group (HFD) ($n = 12$ in each group). NPY-hetero mice (Npy^{+/-}) were used as control ($n = 9$ in each group). In both groups, diethylnitrosamine (DENa) was administered intraperitoneal to induce HCC.

Results HCC was developed in all groups. Generally, HCC progression and fatty infiltration were severer in HFD group than that in RD and CR groups. In Npy^{-/-} mice, the size and number of HCC was the significantly bigger in HFD group than that in RD or CR group. When compared to Npy^{+/-} mice, HCC progression and fatty infiltration were both significantly severe in Npy^{-/-} group than that in Npy^{+/-} group.

Conclusion In Npy^{-/-} mice, the fatty infiltration and HCC progression was significantly severer than that of Npy^{+/-} mice.

P-0896

The importance of MTHFR A1298C and C677T gene polymorphisms in non-obese NASH patients

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Introduction and aim: Our aim in this study is to determine the relationship between the NASH and MTHFR C677T and A1298C gene polymorphisms, especially in non-obese NASH patients.

Material and Method: Eighty-eight NASH patients whose diagnoses were confirmed by liver biopsies and 90 healthy volunteers as control group were included in the study. We investigated MTHFR A1298C and C677T gene polymorphisms and compared NASH patients and controls. NASH patients were assigned to two groups according to whether they are obese.

Results: Eighty-eight NASH patients (52M, 36F, mean age 45 years), and 90 healthy controls (53M, 37F, mean age 41 years) were included in the study. According to BMI values of NASH patients, 55 patients

were non-obese and 33 patients were obese. There was no statistically significant difference between distribution of MTHFR A1298C polymorphism of NASH patients and controls ($p > 0.05$). The proportion of TT genotype of MTHFR C677T polymorphism of NASH patients was significantly higher than that of controls ($p < 0.01$). Also the proportion of TT genotype of MTHFR C677T polymorphism of non-obese NASH patients was significantly higher than that of controls ($p < 0.01$). However, the proportion of TT genotype of MTHFR C677T polymorphism of obese NASH patients was not significantly different than the control group ($p > 0.05$). MTHFR C677T CC (wild) genotype was significantly lower in non-obese NASH patients than controls ($p < 0.05$).

Conclusion: This study revealed that TT genotype of MTHFR C677T polymorphism is more frequent, especially in non-obese NASH patients than in healthy controls. This finding shows that genetic factors are particularly more important in non-obese NASH patients.

P-0897

The role of hematopoietic stem cells in liver pathogenesis

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We have shown that hepatic stellate cells are believed to play a key role in the development of liver fibrosis. Hematopoietic stem and progenitor cells (HSCs), which express CD34 antigen, circulate at a very low frequency in peripheral blood in steady state. HSCs have been believed to differentiate to a variety of cells in certain conditions, however, the role of HSCs in liver diseases and regeneration has not been well elucidated. Therefore we investigated the mobilization of HSCs and differentiation of HSCs in liver mouse models with carbon tetrachloride-induced liver injury, MCD diet and NASH models. And we have transplanted EGFP-marked HSCs into bone marrow after whole body irradiation. We have investigated the numbers of circulating HSCs by flow cytometry. We also examined the differentiation of HSC in mouse liver injury models. The numbers of circulating HSCs increased significantly in NASH models. Mouse model experiments suggested that HSCs contribute to the generation of hepatic stellate cells after carbon tetrachloride-induced injury liver injury and macrophages after MCD diet-induced fatty liver and NASH models. In conclusions, HSCs play an important role in liver fibrosis, inflammation and hepatic pathogenesis. Differentiation of HSCs may be dependent on liver pathological conditions.

P-0898

Characterization of liver myeloid-derived suppressor cells in nonalcoholic fatty liver disease

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Background: Myeloid-derived suppressor cells (MDSCs) are recognized as suppressors of T cell functions. In this study, we characterized the phenotype of liver MDSCs in a murine model of NAFLD and explored the mechanism underlying their immunosuppression.

Methods: C57BL/6 mice were fed a normal diet (ND) or a high-fat diet (HFD). Different subtypes of cells were sorted from liver non-parenchymal cells by FACS. CD11b⁺Gr1⁺ cells were co-cultured with T cells in the presence of anti-CD3 beads and suppressive functions were investigated using a CFSE assay. Nitric oxide (NO) concentrations in culture supernatants were measured using Griess reagent.

Results: In the livers of HFD-fed mice, the frequency of CD11b⁺Gr1^{dim} cells was higher than that in the livers of ND-fed mice. These cells were divided into SSC^{high} and SSC^{low} populations. The frequency of SSC^{low}CD11b⁺Gr1^{dim} cells increased in the livers of HFD-fed mice to a greater extent than that observed in the livers of ND-fed mice. In addition, SSC^{low}CD11b⁺Gr1^{dim} cells suppressed T cell proliferation. We also found that SSC^{low}CD11b⁺Gr1^{dim} cells expressed iNOS after co-culture with T cells, and the supernatants obtained from the co-culture of SSC^{low}CD11b⁺Gr1^{dim} cells with T cells had higher concentrations of NO. By adding iNOS inhibitor to the SSC^{low}CD11b⁺Gr1^{dim} cells and T cells co-culture, T cell proliferation was restored.

Conclusions: SSC^{low}CD11b⁺Gr1^{dim} cells represent authentic monocytic MDSCs in the liver, and their numbers were increased in the livers of NAFLD mice. The suppressive function of MDSCs in NAFLD liver serves an important negative feedback function in liver inflammation.

P-0899

Adipolysis efficacy of aged black garlic extract in 3T3-L1 adipocytes

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This study was to purpose the lipid lowering effect on aged black garlic (ABG) extract solution in adipocyte-like differentiated 3T3-L1 cells. 3T3-L1 pre-adipocytes were seeded to 24-well plates and proliferated to reach confluence and then 0.5 mM Methylisobutylxanthine, 0.5 μ M Dexamethasone, 5 mg/mL insulin were added to the media to induce cell differentiated of adipocytes. ABG treated with media containing 0, 0.625, 1.25, 2.5, 5 and 10 mg/mL for 14 days. After treatments, adipocytes were stained with Oil Red O on Day 7 and 14. The staining results demonstrated that ABG treatments at 2.5, 5 and 10 mg/mL markedly reduced lipid drop size in 3T3-L1 adipocytes on day 7 and 14. As results of ELISA, the ABG for 2.5, 5 and 10 mg/mL significantly reduced lipid accumulation in ratios of 37, 62, 50 % compared to the untreated control group, respectively. In pilot study using db/db mice, we fed the mice a saline or ABG (60 g/kg/day) for 4 weeks. Body weight and HDL-cholesterol in ABG group increased than control group. But, triglyceride decreased in the ABG group compared with the control group. ABG extract reduced lipid drop size in 3T3-L1 adipocytes. Lipid accumulation ratio of the ABG for 2.5 (* $P < 0.05$), 5 (** $P < 0.01$), 10 (* $P < 0.05$) mg/mL were significantly reduced compared with the untreated control group, respectively. In addition, the results of blood biochemistry assay demonstrated that ABG extract increased HDL-cholesterol and decreased triglyceride compared with the untreated control group.

Thus, ABG extract could be contribute to prevent and/or to treat obesity complications.

P-0900

Analysis of the direct interaction between macrophages and microbiota

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Background/aims: It is well known that the interaction between liver macrophages (M Φ , Kupffer cells) and gut microbiota plays an important role in the development of liver diseases including fulminant hepatitis and non-alcoholic fatty liver disease (NAFLD). Fulminant hepatitis is induced by priming with *Propionibacterium acnes* (*P. acnes*) and subsequent challenge with lipopolysaccharide (LPS) in mice. On the contrary, *Clostridium butyricum* (CB) could prevent NAFLD progression and promote IL-10 production by intestinal M Φ . The direct interaction between these bacteria (*P. acnes* or CB) and M Φ remains to be elucidated. In this study we investigated the direct effects of these bacteria on activation of M Φ .

Materials and methods: RAW264.7 cells (mouse macrophage cells) were treated with heat-killed bacteria (*P. acnes* or CB (MIYAIRI 588)) and/or LPS for 6 h, the changes in cytokines (TNF- α , IL-10) and Toll-like receptors (TLR2, TLR4) expression were evaluated by RT-PCR. To evaluate phagocytic capacity, the treated cells were incubated with the Latex-bead-Rabbit IgG-FITC for 24 h, and the fluorescent intensity was measured.

Results: TNF- α expression was up-regulated by not only LPS but also these bacteria in a dose-dependent manner. Each optimal expression of TNF- α was increased at 10.5, 15.4, and 22.9-fold by LPS, *P. acnes*, and CB, respectively. Moreover, *P. acnes* or CB-induced TNF- α expression was drastically increased by addition of LPS. Although TNF- α expression was up-regulated by LPS, the phagocytic capacity was not changed. **Conclusions:** Macrophages could directly interact with bacteria, which could influence on pathogenesis of liver diseases through the innate immune system.

P-0901

Fatty acid-overloaded steatotic hepatocytes display altered phospholipidome and membrane integrity

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Aims: (1) Define the effect of fatty acid overload on hepatocyte phosphatidylcholine (PC) and phosphatidylethanolamine (PE) profiles using lipidomic techniques. (2) Identify the metabolic pathways leading to changes in the phospholipidome upon fatty acid treatment

using flux analysis. (3) Identify mechanisms which link fatty acid overload, changes in the hepatocyte phospholipidome and NAFLD.

Methods: Rat primary hepatocytes were treated with 0–2 mM fatty acid (2:1 oleate and palmitate) for up to 48 h. The hepatocyte lipidome was analysed by liquid chromatography mass spectrometry. Flux analysis with D9-choline, D3-methionine, and D4-ethanolamine was used to label phospholipids produced via specific pathways. To interrogate possible mechanisms behind the change in the phospholipidome and NAFLD, cell membrane PE was detected with PE-specific dye, and cell membrane permeability measured with propidium iodide.

Results: Under FA treatment, we observed a decrease in hepatocyte PC/PE driven mainly by the increment in cellular PE. Flux analysis indicated an increased production of PE via CDP-ethanolamine pathway drives the increase in cellular PE leading to a lower PC/PE. Both PC and PE fatty acid profiles reflected the oleic acid and palmitic acid supplementation. We observed an increase in plasma membrane PE content and this was associated with an increase in membrane permeability and the loss of membrane integrity.

Conclusion: Our study suggests that fatty acid overload alters the hepatocyte phospholipidome leading to a loss of membrane integrity. This may be a major contributor to the lipotoxic effect seen in NAFLD.

P-0902

Fermented soymilk prevent free fatty acid-induced lipogenesis in hepatocellular steatosis model

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Introduction: Ingredients of soy and fermented products have been widely utilized as food supplement for health-enhancing properties, such as reducing the risk of osteoporosis, protection of cardiovascular diseases, and prevention of prostate and breast cancer. This study was carried out to examine the effects of fermented soymilk (FSM) on the free fatty acid-induced lipogenesis in an in vitro model of hepatocellular steatosis model.

Materials and methods: HepG2 cells were incubated with 0.2 mM of palmitic acid (PA) for 24 h to induce lipogenesis and to accumulate the intracellular lipid accumulation, which was observed by oil red O and Nile red staining. The PA treated cells were co-incubated with 0.04–1.0 % of lyophilized FSM, 0.05 mM of genistein, and 50 nM of estrogen, respectively.

Results: Lipid accumulations in the PA and FSM co-incubated cells were significantly decreased by 0.5 and 1.0 % of FSM without cytotoxicity. Treatments of PA and combining with genistein and estrogen significantly increased the expressions of SREBP-1. However, FSM co-incubation significantly attenuated the expression of SREBP-1 in the PA treated cells. In addition, expression of NRF-2 and phosphorylation of ERK were significantly increased in the PA and FSM co-incubated cells. PA induced ROS production was significantly reduced by 1.0 % of FSM. Meanwhile, genistein or estrogen alone did not lead to significant differences in ROS production.

Conclusion: Our results show that bioactive components, except genistein and phytoestrogen, in fermented soymilk protect hepatocytes against lipid accumulation and ROS production induced by free fatty acid.

P-0903

Gut Microbiota and their metabolic influence in neonatal intestine by early colonization

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Objectives and study: To see the metabolic and growth promoting effect of intestinal microflora on neonatal mice.

Methods: Naturally inhabiting commensal intestinal bacteria were isolated from mouse fecal samples and taxonomically classified through morphological observation, biochemical typing, and/or 16S rDNA typing. The isolated Probiotics, Bacteroidetes, Firmicutes, or a combination of the Bacteroidetes and Firmicutes groups (B/F) were fed to germ-free (GF) neonatal mice immediately after birth, and the effect on growth was monitored periodically by measuring the change in body weight.

Results: The immediate colonization of neonatal mice with the Bacteroidetes, Firmicutes, or combined groups resulted in an increased gain in body weight compared to the non-colonized, GF controls. The Firmicutes group of bacteria most significantly increased the body weight of neonatal mice compared to GF control [34.55 + 0.86 g (Firmicutes) versus 27.7 + 0.88 g (GF); n = 13–15; p 0.05]. Unexpectedly, the colonization with a group of probiotics bacteria was fatal to the neonates. These results suggest that the immediate intestinal colonization of low birth weight infants with the Firmicutes group of bacteria could be an ideal therapeutic treatment for boosting proper development and growth of the infants.

Conclusion: In conclusion, these studies are showing that the Firmicutes group of bacteria has an excellent potential as a therapeutic agent for weight gain of neonates but application of probiotics in an attempt to activate weight gain of neonate should be reconsidered.

P-0904

Identification of SENP3 as a potential modulator in the lipid metabolism pathway in NAFLD

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Aim: The aim of this study was to investigate if and how SENP3 was involved in non-alcoholic fatty liver disease (NAFLD). **Method:** Liver biopsy samples of NAFLD patients and normal controls, obtained from Shanghai Ruijin Hospital, were subject to immunohistochemical staining of SENP3 expression. Meanwhile, Sprague Dawley (SD) rats fed with high-fat diet were utilized as NAFLD animal model to further validate the results found in human. Finally, we treated normal liver cell line (L02) with medium containing oleate and palmitate (oleate/ palmitate, 2:1 ratio) as the cell model of NAFLD to explore the potential mechanistic link between SENP3 and NAFLD. SENP3 was ectopically overexpressed or silenced in L02, and determined the

cellular lipid content by Oil Red O staining. Furthermore, mRNA-sequencing analysis was performed in six cell RNA samples, including L02, L02/ FFA (0.5 mmol/L, 12h), SENP3-silenced-L02, SENP3-silenced-L02/FFA (0.5 mmol/L, 12h), SENP3-overexpressed-L02, SENP3-overexpressed-L02/FFA (0.5 mmol/L, 12 h).

Conclusion: Elevated expression of SENP3 was observed in the NAFLD than controls. Furthermore, SENP3 overexpression promoted lipid accumulation, while the ablation of it inhibited lipid accumulation. Bioinformatics analysis of mRNA-sequencing results predicted 11 genes associated with SENP3 in the model involved in the lipid metabolism pathway. Taken these findings together, we report for the first time that upregulated expression of SENP3 in the liver plays an important role in the progression of NAFLD, especially in regulating lipid metabolism pathway. The potential underline mechanism is still under investigated

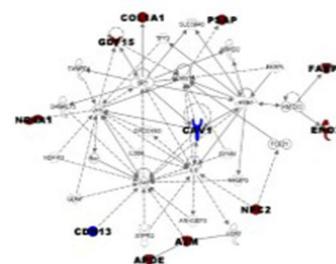


Figure 1. Bioinformatics analysis result of mRNA-sequencing.

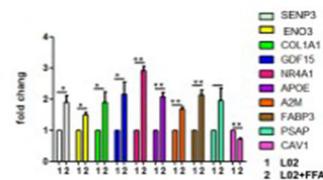


Figure 2. The validation of the bioinformatics analysis result in cell models with Real-time RT-qPCR. (*P<0.05, **P<0.01)

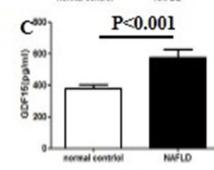
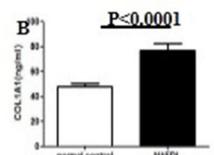
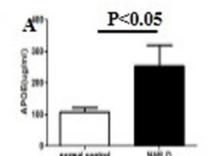


Figure 3. Serological detection of APOE (A), COL1A1 (B), and GDF15 (C) in 40 normal controls and 50 NAFLD patients.

P-0905

Protective role of cytoglobin in liver inflammation and fibrogenesis in mouse steatohepatitis model

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Objective: To clarify the role of cytoglobin (CYGB) in the development of liver inflammation and fibrosis in a mouse model of non-alcoholic steatohepatitis.

Methods: A mouse steatohepatitis model was generated in wild type (WT), *Cygb*^{-/-} (KO), *Cygb*-over-expressing (TG) mice by feeding a choline deficient L-amino acid-defined diet (CDAA) for 16 weeks.

These mice were analyzed for histopathology and gene expression profiles.

Results: CDAA treatment for 16 weeks induced robust inflammation, fibrosis, and prominent expression of γ -H2AX, a marker of oxidative stress-related DNA double-stranded breaks, in *Cygb*^{-/-} livers compared to WT. TG mice were found to be resistant to inflammatory reaction. An imbalance between oxidative stress and antioxidant defense in KO mice fed CDAA was demonstrated by the altered expression of 31 oxidative stress-related genes, including myeloperoxidase (50-fold against WT mice, $p = 0.002$) and glutathione peroxidase 2 (5-fold, $p = 0.004$), both of which are involved in H₂O₂ production. In addition, *Cygb*^{-/-} mice exhibited induction of nitric oxide synthase, high levels of nitrotyrosine formation, and an increased number of hypoxic (pimonidazole-staining-positive) hepatocytes. In contrast, *Cygb*-over expression protected mice from CDAA induced liver inflammation and fibrosis via suppressed oxidative stress conditions.

Conclusion: These results indicate that CYGB may control oxidative stress in chronically inflamed liver, which provides important insights into the role of CYGB expressed in HSCs in liver homeostasis, fibrosis and cancer development.

P-0906

Reliability of transient elastography for the detection of fibrosis in severely obese patients

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Aims: We evaluated the diagnostic value of transient elastography (TE) with FibroScan[®] in diagnosing bariatric surgery patients with severe fibrosis/cirrhosis.

Methods: TE using the XL probe was performed peri-operatively in 110 consecutive patients who underwent bariatric surgery with liver biopsy. Pre-operative demographic data and liver stiffness measurement (LSM) were compared to liver biopsy staging. A LSM of >7.9 kPa was used as the cut-off, predictive of severe fibrosis/cirrhosis.

Results: A valid LSM was obtained in 73/110 (66.4 %). Liver biopsy showed 7 patients with advanced fibrosis/cirrhosis (F3–4). TE was predictive in 2/7 (28.6 %) patients while three (42.8 %) patients had invalid LSM and 2 (28.6 %) patients had LSM <7.9 kPa. F3–4 patients had higher waist:hip ratio (1.02 vs. 0.92; $p = 0.01$), lower platelet count (214 vs. $290 \times 10^9/L$; $p = 0.002$) and higher GGT (130 vs. 54U/L; $p = 0.002$). Metabolic risk factors, liver synthetic function and ALT were not predictive of fibrosis stage. 23/68 (33.8 %) patients with F0–2 fibrosis had false positive LSMs. At a cut-off value of 7.9 kPa, the sensitivity, specificity, positive and negative predictive values for F3–4 disease were 50, 66, 8, and 96 % respectively. All 9 patients (8.2 %) with body mass index (BMI) <35 kg/m² had LSMs concordant with the liver biopsy staging. **Conclusions:** TE underestimated fibrosis or did not provide a valid reading in 71.4 % of patients with severe fibrosis/cirrhosis. The severity of obesity is likely to increase the discordance of TE. In patients with a BMI >35 kg/m², transient elastography measurement should be interpreted with caution.

P-0907

Comparison of FibroTouch and FibroScan for the assessment of liver steatosis in NAFLD patients

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Background and aim: The controlled attenuation parameter (CAP) implemented in FibroScan is reported to be a noninvasive means of detecting steatosis. However, there were some limitations. We therefore performed a head to-head comparison between FibroTouch and FibroScan.

Methods: A total of 188 patients with non-alcoholic fatty liver disease (NAFLD) were enrolled for study between January 2014 and August 2015, 26 of which underwent liver biopsy. The patients underwent FibroScan testing (group A) and FibroTouch testing (group C), after which the operator examined a timemotion ultrasound image from the FibroScan test and located a specific liver portion for focused FibroTouch testing (group B). The consistency between the two tests results was investigated by Pearson S correlation analysis.

Results: The Pearson correlation were all >0.8 ($P < 0.05$) and there was no statistically significant difference found between the results from FibroScan and FibroTouch. The rates of successful detection were 100 % for FibroTouch and 92 % for FibroScan.

Conclusions: FibroTouch presented excellent diagnostic performance for severe steatosis with high sensitivity and specificity in Chinese patients with NAFLD, and has a higher rate of successful detection than FibroScan.

Keywords: Non-alcoholic fatty liver disease; Controlled attenuation parameter; FibroTouch; FibroScan

P-0908

Usefulness of the FibroScan[®] XL probe in patients with nonalcoholic fatty liver disease

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Background: A novel probe of FibroScan[®] XL probe is designed specifically for obese patients. However, no comparative study exist for clinical usefulness among the XL probe, standard M probe and Virtual Touch Quantification[®] (VTQ) in patients with nonalcoholic fatty liver disease (NAFLD). The aim of this study was to evaluate the feasibility of the XL probe compared with the other two modalities.

Methods: A total of 540 consecutive NAFLD patients were prospectively enrolled. Liver stiffness measurements (LSMs) were evaluated with VTQ and FibroScan M and XL probes simultaneously,

the quality of which was categorized as inadequate (success rate <60 % and/or interquartile range/median value \geq 30 %).

Results: Inadequate LSM rate with the XL probe was significantly lower than those with the M probe and VTQ (XL probe, 21.3 %; M probe, 30.9 %; VTQ 32.4 %; $P < 0.0001$). In multivariate analysis, skin capsule distance (SCD) was strongly associated with inadequate rates with the M probe and VTQ. A total of 20 liver biopsy specimens confirmed diagnostic accuracy and high applicability of the XL probe. In patients with long SCD, the XL probe showed better diagnostic performance than the M probe, because the LSM of M probe tended to be overestimated. Furthermore, in cirrhotic patients, the XL probe showed an advantage in performance over VTQ, the LSM of VTQ was underestimated.

Conclusions: The FibroScan XL probe is a feasible modality for the assessment of liver fibrosis in NAFLD patients with long SCD, showing a better diagnostic performance than conventional M probe and VTQ.

P-0909

Novel quantitative assessment of liver fibrosis in NAFLD

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Introduction: Digital image analysis on stained liver biopsy has previously shown a significant correlation of collagen proportionate area (CPA) with traditional semi-quantitative staging systems. However, histological staining used may introduce operator-dependent variation and collagen progression dynamics have not been taken into consideration. The new second harmonic generation (SHG), two-photon excited fluorescence (TPEF) microscopy can be used to observe collagen and hepatocyte morphology without staining. Morphological features of collagen can be extracted by sophisticated image analysis algorithms.

Methods: Liver biopsies from 101 patients with NAFLD were staged according to the NASH CRN and with the seven category Ishak staging system modified to be appropriate for NASH. Quantitative analysis was done on the unstained biopsy samples using SHG/TPEF microscopy to generate 100 quantitative collagen features including overall, portal, septal and fibrillar collagen in liver tissue. For comparison, digitized images of Sirius red stained sections were also acquired to calculate the CPA. The SHG collagen features were compared with CPA on the correlation with both staging systems and clinical data of the patients.

Results: Both staging systems of NASH fibrosis were more significantly correlated with SHG collagen features (NASH CRN: $r = 0.68$, $p < 0.001$; Ishak: $r = 0.74$, $p < 0.001$) than with CPA using staining (NASH CRN: $r = 0.48$, $p < 0.001$; Ishak: $r = 0.54$, $p < 0.001$); similar results were found correlating SHG collagen features with CPA with clinical data (e.g. SHG and ALT: $r = 0.40$, CPA and ALT: $r < 0.3$). **Conclusions:** Compared with traditional stained image analysis, quantitative assessment using SHG collagen features recorded by SHG/TPEF microscopy is more robust for evaluating fibrosis in NAFLD.

P-0910

Utility of heartbeat-induced strain elastography (S-Map) in staging fibrosis in NASH

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Objectives: We investigated the utility of heartbeat-induced strain elastography (S-Map) in staging fibrosis in nonalcoholic steatohepatitis (NASH).

Methods: Subjects were 22 patients with nonalcoholic fatty liver disease (NAFLD) who underwent liver biopsy and S-Map in the last 2 years. Ultrasound (US) was performed from the right intercostal space using LOGIQ E9 XDclear (GE Healthcare) and a 3.5-MHz convex probe (C1–6). S-Map measurement was performed according to the manufacturer's specification to obtain images coinciding with the heartbeat. S-Map measurement was repeated 5–7 times to calculate the mean measurement value. The mean measurement value was multiplied by the distance between ROI and the heart (HD) to calculate SHD for assessing the correlation with fibrosis staging in the liver biopsy. Receiver operating characteristic (ROC) curves were generated to evaluate the diagnostic performance of SHD in fibrosis staging.

Results: Compared with patients with fibrosis 0–3, those with fibrosis 4 had a significantly lower SHD ($P = 0.0032$) and they therefore had hard liver tissue. With respect to the diagnostic performance of SHD in patients with fibrosis ≤ 4 , the area under the ROC curve was 0.92, cutoff value was 122, sensitivity was 87.5 %, and specificity was 83.3 %.

Summary: Although further study is needed to increase the number of patients, the findings of this study suggest the utility of S-Map strain index in staging fibrosis in NASH.

P-0911

S-Allyl cysteine improves fatty liver disease and diabetes in Otsuka Long-Evans Tokushima Fatty rats

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There is a very high prevalence of nonalcoholic fatty liver disease (NAFLD) in individuals with type2 diabetes mellitus. Furthermore, accumulation of lipids in the liver and skeletal muscle lowers insulin activity, thereby causing impaired glucose tolerance. NAFLD and diabetes may be established as a significant risk factor of hepatocellular carcinoma. It is important to prevent and improve NAFLD and diabetes in a safe and low-cost manner. S-Allyl cysteine (SAC), an aged garlic extract with antioxidant activity, was investigated to determine whether SAC can improve fatty liver and diabetes in Otsuka Long-Evans Tokushima Fatty rats (OLETF), a type 2 diabetes rat model. Male OLETF and age-matched Long-Evans Tokushima

Otsuka rats (LETO) were used. Body weight, blood glucose levels, and hemoglobinA1c (HbA1c) were all checked at 29 weeks of age, and the OLETF and LETO were divided into two groups (control and SAC group). SAC (0.45 % in diet) was administered to rats between 29 and 42 weeks of age. Rats were killed at 43 weeks of age, and detailed analyses were performed. SAC improved HbA1c, blood glucose, triglyceride, and low-density lipoprotein cholesterol levels. Furthermore, SAC normalized plasma insulin levels. SAC activated the mRNA and protein expression of both PPAR -alpha and -gamma, as well as inhibiting PDK4 in OLETF liver. SREBP-1c and FoxO1 proteins were normalized by SAC in OLETF liver. In conclusions, these findings may support the hypothesis that SAC has diabetic and NAFLD therapeutic potential as a potent regulating agent against lipogenesis and glucose metabolism.

P-0912

Anti-fibrotic effect a novel, non-bile acid FXR agonist in a model of non-alcoholic steatohepatitis

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The Farnesoid X Receptor (FXR) is a bile acid (BA)-activated nuclear receptor and a key regulator of bile acid metabolism. FXR agonists decrease hepatic triglyceride synthesis leading to reduced steatosis; inhibit hepatic stellate cell activation reducing liver fibrosis; improve gut barrier function which reduces hepatic inflammation and increase FGF19 leading to improved hepatic insulin sensitivity. In clinical studies, obeticholic acid (OCA), a bile acid-derived FXR agonist, showed efficacy in both Primary Biliary Cirrhosis (PBC) and non-alcoholic steatohepatitis (NASH) patients; however OCA treatment was associated with increased pruritus. Here we describe a novel, non-bile acid FXR agonist GNF5120 which showed high potency on FXR with no activity on other nuclear receptors or the bile acid activated G-protein coupled receptor TGR5. GNF5120 effectively induced FXR target genes in primary human hepatocytes and in vivo in the intestine and liver of rats. In a mouse model of NASH (the Stelic STAM model), GNF5120 decreased hepatic fibrosis and all parameters of the NAS score including steatosis, inflammation and ballooning. These preclinical data describe a novel, non-bile acid derived FXR agonist GNF5120 with the potential for strong efficacy in PBC and NASH.

P-0913

Obese insulin resistant diet-induced NASH (DIN) mouse as a model for evaluating drugs targeting NASH

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Background: Several animal models used for evaluating drugs targeting non-alcoholic steatohepatitis (NASH) do not exhibit obesity and insulin resistance. Our aim was to develop a diet-induced NASH

(DIN) mouse model that would integrate human metabolic syndrome characteristics.

Methods: C57BL6/J mice were fed a control chow with normal tap water (CC) or a high fat (60 %)/high cholesterol (2 %) diet (DIN) with tap water supplemented with fructose (10 %) for 16 weeks. Reference drugs ezetimibe, pentoxifylline or telmisartan administered through the diet for 16 weeks were evaluated.

Results: Compared to CC mice at 16 weeks of diet, DIN mice were obese (CC: 31 ± 0.6 g; DIN: 45.4 ± 1.8 g, p < 0.001) and insulin resistant with a 6-fold higher HOMA-IR index (p < 0.001). Plasma ALT and AST levels were 410 and 90 % higher, respectively (both p < 0.001 vs. CC). Hepatic triglycerides, fatty acids and cholesterol levels were strongly increased (all p < 0.001 vs. CC). Hepatic expression of genes involved in oxidative and endoplasmic stress, lipogenesis and fibrosis was significantly higher in DIN mice, while histology analysis and NAS scoring indicated strong hepatic steatosis, hepatocyte ballooning and fibrosis (total NAS score of 7.3 over 12). In DIN mice, ezetimibe and pentoxifylline tended to improve liver complications. However, telmisartan was the most effective drug in preventing liver fibrosis and metabolic parameters, suggesting a different mode of action.

Conclusion: Our data indicate that the DIN mouse model replicates NASH in the context of metabolic syndrome, and is therefore a relevant model for evaluating and differentiating drugs targeting NASH.

P-0914

P2Y13 receptor agonist CER-209 decreases both atherosclerosis and liver steatosis in vivo

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Background: Non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH) are components of the growing worldwide epidemic of obesity/diabetes. Among recent AASLD guidelines, treatment of hyperlipidemia to lower lipids through regulation of LDL was recommended. In contrast to the LDL pathway, the reverse lipid transport (RLT) driven by HDL particles transfer cholesterol from non-hepatic cells to the liver where it is excreted from the body in the form of bile acids and biliary cholesterol. The capacity of the HDL particles to mobilize cholesterol from atherosclerotic plaques confers them a protective effect against heart disease. The increase of bile acids and cholesterol elimination by the liver under the direction of HDL has never been studied in NAFLD and NASH pathologies. We have recently described a new class of P2Y13 receptor agonist (CER-209), which by enhancing the number of HDL particles in mice have a very strong negative impact on the development of atherosclerotic plaques.

Methods and results: Using a high-cholesterol diet rabbit model, CER-209 (30 µg/kg/day for 4 weeks) increased the number of and functionality of HDL particles (as measured by cholesterol efflux capacity) and, thus, decreased atherosclerotic plaque development (−30 %). Importantly, we also observed, for the first time, the impact of CER-209 on liver steatosis from the same treated animals where it was associated with decreases in hepatic triglyceride and cholesterol levels (−50 and −53 % versus placebo, respectively).

Conclusion: CER-209 decreases atherosclerotic plaque development and liver steatosis in an in vivo animal model.

P-0915

P2Y₁₃ receptor agonist CER-209, an anti-atherosclerotic compound, decreases liver steatosis in vivo

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Background: Non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH) are increasing as a consequence of growing worldwide obesity/diabetes. NAFLD is the most prevalent chronic liver disease, affecting 20–40 % of the general population, and approximately one-third of patients with NAFLD will progress to NASH. High-density lipoprotein (HDL) metabolism is well established as being inversely correlated to atherosclerosis in humans promoting lipid elimination through the reverse lipid transport.

Methods: We used a new in vivo mouse model of NAFLD/NASH induced using high fat/cholesterol/cholesterol diet over a short-term period to determine whether CER-209, a new compound designed to improve HDL metabolism (i.e. HDL elimination), could also have a marked effect on liver metabolism. A 1-week induction period with CER-209 was followed by 2 weeks of treatment at doses ranging between 0.2 to 3 mg/kg/day.

Results: In this short term model, the overall steatohepatitis was markedly reduced by CER-209 as determined by hepatic lipid measurement (−18, −25 and −13 % for cholesterol, triglycerides and fatty acids versus placebo, respectively). Furthermore, the measurement of the ALT/AST enzymes in the plasma was also markedly decreased (−50 % for ALT) at doses as low as 0.2 mg/kg/day.

Conclusion: It is anticipated that CER-209 has considerable potential for treating the pathophysiology of NASH due to its ability to specifically target the pathways for cholesterol elimination without the pleiotropic effects characteristic of drugs working through nuclear factors, such as PPAR and FXR agents.

P-0916

Sodium alginate prevents NASH progression and liver carcinogenesis in obese and diabetic mice

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Background and aim: Obesity and related metabolic abnormalities play a key role in liver carcinogenesis. Non-alcoholic steatohepatitis (NASH), which is often complicated with obesity and type 2 diabetes mellitus (T2DM), is associated with hepatocellular carcinoma (HCC) development. Sodium alginate (SA), which is extracted from brown seaweeds, is marketed as a weight loss supplement due to its high viscosity and gelling properties. In the present study, we examined the effects of SA on the progression of NASH and NASH-related liver

carcinogenesis in monosodium glutamate (MSG)-treated mice, which show obesity, T2DM and NASH-like histopathological changes.

Method: Male MSG-treated mice were given intraperitoneal injection of diethylnitrosamine at 2 weeks of age, and thereafter they received a basal diet containing 5 % of high- or low-molecular SA throughout the experiment (16 weeks). Results; MSG-treated mice fed basal-diet showed significant obesity, hyperinsulinemia, hepatic steatosis and liver tumor development. SA administration suppressed body weight gain, improved insulin sensitivity and hyperleptinemia, attenuated the inflammation in both the liver and white adipose tissue, and inhibited hepatic lipogenesis and the progression of NASH. SA also reduced oxidative stress and increased anti-oxidant enzymes in the liver. The development of liver tumors, including liver cell adenoma and HCC, was significantly inhibited by SA supplementation.

Conclusion: Oral SA supplementation improves liver steatosis, insulin resistance, chronic inflammation, and oxidative stress, which lead to preventing the development of liver tumorigenesis in obese and diabetic mice. SA may have ability to suppress steatosis-related liver carcinogenesis in obese and diabetic subjects.

P-0917

Echocardiographic findings in patients with non-alcoholic fatty liver disease

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Aim: To compare the echocardiographic findings in patients with non-alcoholic fatty liver disease (NAFLD) with control group.

Material-methods: Totally 49 patients with the diagnosis of biopsy proven NAFLD and 20 cases without any fatty infiltration in ultrasound were compared.

Results: Table 1. Echocardiographic findings of 2 groups NAFLD (n:49) CONTROL (n: 20) pCarotid intima/media thickness 0,98 ± 0,01 0,78 ± 0,01 0,01 Epicardial fat thickness 0,19 ± 0,06 0,15 ± 0,07 0,09 Interventricular Septum thickness in diastole (cm) 0,99 ± 0,10 0,97 ± 0,09 0,42 Left ventricular end diastolic diameter (cm) 4,29 ± 0,48 4,10 ± 0,37 0,27 Posterior wall thickness in diastole (cm) 0,99 ± 0,09 0,98 ± 0,07 0,67 Interventricular septum thickness in systole (cm) 1,55 ± 0,18 1,24 ± 0,08 0,01 Left ventricular end systolic diameter (cm) 2,87 ± 0,34 2,73 ± 0,29 0,27 Posterior wall thickness in systole (cm) 1,50 ± 0,18 1,45 ± 0,10 0,41 Ejection fraction (%) 61,5 ± 2,86 62,5 ± 3,76 0,37 Left atrium diameter(cm) 3,28 ± 0,35 2,79 ± 0,37 0,04 Aort diameter (cm) 3,07 ± 0,30 2,97 ± 0,36 0,37 LA/Ao 1,06 ± 0,12 0,90 ± 0,31 0,01 Interventricular relaxation time (sec) 0,09 ± 0,12 0,08 ± 0,01 0,67 Interventricular contraction time (sec) 0,07 ± 0,02 0,07 ± 0,02 0,78 Ejection time (sec) 0,27 ± 0,04 0,28 ± 0,03 0,36 TEI 0,58 ± 0,08 0,56 ± 0,09 0,66.

Conclusion: Increased carotid/intima media thickness and left atrium diameter is associated with cardiovascular diseases in long term in NAFLD patients.

P-0918

Stability of liver proton density fat fraction and changes in R2* Induced by Gd-EOB-DTPA

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Purpose: The purpose of this study was to assess changes in liver proton density fat fraction (PDFF) and R2* measurements in the presence of changes in tissue relaxation times induced by administering gadoxetate disodium at 3T MRI.

Materials and methods: Forty-five patients were imaged at 3T with chemical-shift based MRI sequence before and 20 min after administration of gadoxetic acid. Image reconstructions were performed to obtain PDFF and R2* images. A single radiologist measured PDFF and R2* values on precontrast and postcontrast images. Precontrast and postcontrast PDFF values were compared using intraclass correlation coefficient (ICC), linear regression, and Bland-Altman analysis. Changes in R2* values from precontrast to postcontrast were correlated with relative liver enhancement (RLE) based on signal intensities on T1-weighted images using Spearman's rank correlation.

Results: The PDFF values were similar between precontrast (8.2 %) and postcontrast (7.9 %) images (ICC = 0.99, linear regression slopes = 0.98, mean difference = -0.31 %), although the R2* values on postcontrast images (54.6 sec⁻¹) increased compared with those on precontrast images (45.8 sec⁻¹). PDFF measurements were stable between precontrast and postcontrast images. Changes in R2* values were correlated with RLE ($p < 0.001$, $r = 0.71$).

Conclusion: PDFF measurements are stable in the presence of changes in tissue relaxation times after administering gadoxetate disodium at 3T MRI. Changes in R2* values correlate with established measures of gadoxetate disodium uptake based on T1-weighted images.

P-0919

New effect and mechanism of Protopanaxadiol in improving nonalcoholic fatty liver disease

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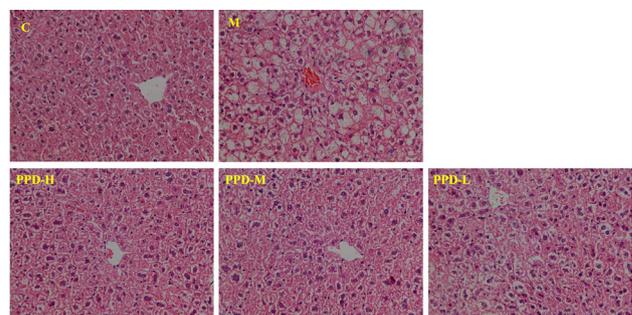
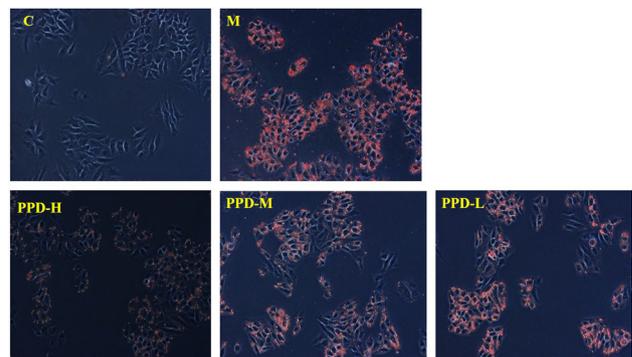
Objective: This study aims to investigate the effect of protopanaxadiol (PPD), one natural compound from Chinese medicine, on nonalcoholic fatty liver disease (NAFLD) and explore the corresponding mechanism.

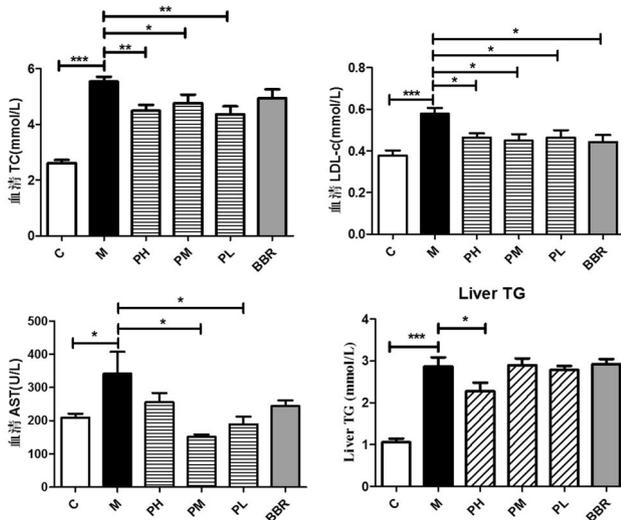
Methods: HepG2 cell was incubated with free fatty acid for 24h to induce in vitro NAFLD model. Three doses of PPD (50, 5, 0.5 $\mu\text{mol/L}$) were then applied to steatotic cells for another 24 h. Oil red O stain

and cell lipid content were used to evaluate its effect. For in vivo model, Male C57BL/6 mice were fed with high fat diet for 3 weeks. Then NAFLD mice were administered intragastrically with PPD (40, 10, 2.5 mg/kg/day) and berberine (100 mg/kg/day) respectively for 4 weeks. Liver histopathology was observed through HE and Oil red O staining. Serum biochemical examination was carried out. And the indices concerning lipid metabolism and oxidative stress were measured.

Results: PPD obviously reduced lipid droplet in HepG2 cells induced by fatty acid. The high dose had the strongest effect (Figure 1). Severe hepatic steatosis, increased triglycerides content, and elevated serum lipid and AST were demonstrated in HF diet-induced mice. PPD reversed the pathologic and serum change (Figure 2, 3). PPD could significantly increase AMPK activation and decrease ACC activation to modulate the lipid metabolism related gene expression, such as FAS, ACOX1, and PPAR-alpha. PPD decreased ROS and HNE levels in steatotic HepG2 cells. The compound could down-regulate CYP2E1 expression.

Conclusion: PPD demonstrates obvious effect on NAFLD models in a dose dependent manner. And the underlying mechanism might include regulating lipid metabolism and oxidative stress





P-0920

The antifibrotic effects of endothelin type A receptor antagonist in NASH model FLS-ob/ob mice

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Background and aim: Endothelin (ET) is a potent vasoconstrictor and can activate hepatic stellate cells, resulting in progress of hepatic fibrosis. Ambrisentan is ET type A receptor antagonist. The aim of the present study is to examine the effects of ambrisentan on hepatic fibrosis in FLS-ob/ob mice.

Methods: FLS-ob/ob mice (12 weeks old) were divided into ambrisentan (5 mg/kg per day) group (n = 8) and control group (n = 5). Ambrisentan was administered orally for the 4 weeks. Hepatic steatosis, fibrosis and inflammation were compared between the two groups.

Results: Body weight and liver weight did not significantly differ between the two groups. Hepatic hydroxyprolin content was significantly lower in the ambrisentan group than in the control group. Areas of α -SMA positivity and hepatic fibrosis estimated by Sirius red staining were also significantly decreased in the ambrisentan group compared with control group. Levels of RNA expression for TIMP-1 in the liver was significantly lower in the ambrisentan group. RNA expression for TGF- β 1, CTGF and collagen-1 had no significant difference in the two groups. Inflammation related mRNA expression did not significantly differ between the two groups. Steatosis related mRNA expression and hepatic steatosis estimated by Oil red staining did not significantly differ between the two groups. ET related mRNA in the liver also did not significantly differ between the two groups.

Conclusions: Ambrisentan attenuated the progression of hepatic fibrosis by inhibiting the activation of hepatic stellate cells and

reducing TIMP-1 expression but did not effect the inflammation and steatosis.

P-0921

Impaired liver functions in patients with diabetes mellitus in Bangladesh

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Aims/objectives: Diabetes mellitus (DM) represents one of the major life style-related pathological conditions. DM is usually found to be associated with obesity, coronary diseases and cerebral pathologies. However, more insights are required to evaluate a temporal relation between DM and hepatic functions. The study presented here was accomplished to assess liver dysfunctions in DM patients.

Materials and methods: A total of 100 patients with type 2 DM and 100 normal healthy controls were enrolled in this study. Different parameters of liver function tests were measured in patients of these two groups.

Results: The levels of bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), albumin and prothrombin time were significantly different in DM patients compared to control subjects. Serum alkaline phosphate level was higher in type 2 DM patients than in control subjects. The prevalence of abnormal values of serum bilirubin, ALT, AST, prothrombin time, and albumin were 5.17, 31.03, 5.17, 5.17, 43.10 and 10.34 %, respectively in type-2 DM patients and 00, 02, 00, 02, 03 and 00 %, respectively in control subjects indicating high prevalence of DM patients with abnormal liver functions.

Conclusion: Abnormal liver functions have been found in type 2 DM patients and this should be considered during management of DM patients.

P-0922

Histological severity and clinical outcomes of non-obese non-alcoholic fatty liver disease

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Objectives: Little is known about nonalcoholic fatty liver disease (NAFLD) in non-obese patients. We studied the severity of NAFLD,

the factors associated with severity and the prognosis in non-obese patients.

Methods: Consecutive patients who underwent liver biopsy for NAFLD were recruited. We used the NASH-CRN system to score the histology. The Asian body mass index (BMI) cutoff of 25 kg/m² was used to define non-obese NAFLD.

Results: Among 307 recruited NAFLD patients, 72 (23.5 %) were non-obese. Despite a similar prevalence of NASH in obese and non-obese patients (51.9 % vs. 43.5 %; $p = 0.217$), obese patients had higher NAFLD activity score (3.8 ± 1.2 vs 3.3 ± 1.3 ; $p = 0.019$), contributed by steatosis (2.0 ± 0.8 vs 1.7 ± 0.8 ; $p = 0.014$) and presence of hepatocyte ballooning (73.4 vs 60.9 %; $p = 0.045$). By ordinal regression in all patients, higher BMI and haemoglobin A1c levels (HbA_{1c}) were independently associated with higher NAFLD activity score. 12 of 18 (66.7 %) non-obese patients with HbA_{1c} ≥ 7 % had NASH, compared to only 16 of 42 (38.1 %) of those with HbA_{1c} < 7 % ($p = 0.042$). Obese patients also had more severe fibrosis (mean fibrosis stage 1.7 ± 1.4 vs 1.3 ± 1.5 ; $p = 0.004$); in non-obese patients, higher creatinine levels was the only predictor of advanced fibrosis. After a median follow-up of 49 months, only 6 (2.0 %) patients died, who were all obese.

Conclusions: Patients with non-obese NAFLD tend to have less severe histological severity and fibrosis and may have a better prognosis than obese patients. Liver biopsy and further investigation are warranted for non-obese patients with HbA_{1c} of 7 % or greater.

P-0923

Major depressive disorder adversely affect treatment outcomes of non-alcoholic fatty liver disease

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Background and aim: Major depressive disorder (MDD) is an important public-health problem, and it is often comorbid with many chronic diseases. The purpose of this study is to identify the clinical features of non-alcoholic fatty liver disease (NAFLD) patients comorbid with MDD, and to investigate the influence of MDD on the therapeutic effect of NAFLD.

Methods: A total of 258 patients with biopsy-proven NAFLD were included. MDD was diagnosed according to the criteria of the *Diagnostic and Statistical Manual of Mental Disorders*, 4th edition, text revision. The patients were followed up for 48 weeks under standard care for NAFLD, which consisted mainly of lifestyle modification.

Results: NAFLD patients comorbid with MDD ($n = 32$) were characterized by more severe histological steatosis and higher NAFLD activity score, and also significantly higher levels of serum aminotransferase, GGT and ferritin, than age-and-sex matched NAFLD patients without MDD. After the 48 weeks of standard care, major parameters such as body mass index, ALT, GGT, and ferritin were significantly improved in NAFLD patients without MDD. However, no significant improvement of these parameters was observed in NAFLD patients with MDD. Particularly, NAFLD patients with unstable MDD (not in full/partial remission) showed severe resistance to the treatment.

Conclusion: This is the first study to assess the clinical features of NAFLD patients comorbid with MDD. All hepatologists should recognize the fact that comorbid MDD adversely affect treatment outcomes for NAFLD. It is required to develop new lifestyle

modification programs that enable NAFLD patients with MDD to achieve the treatment goal.

P-0924

NAFLD in Type 1 diabetes mellitus: prevalence and association with metabolic syndrome and CVD

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Significance: Nonalcoholic Fatty Liver Disease (NAFLD) in type 2 diabetics is an established independent factor in increased CV mortality. The significance of NAFLD in Type 1 DM has not been fully discussed. Our group aims to determine the prevalence of NAFLD in patients with T1DM and its association with CVD.

Methodology: This prospective cross-sectional study included adult Type 1 DM patients at the Philippine General Hospital. Patients with significant alcohol history and other conditions that may cause steatosis were excluded. Demographics, laboratory, hepatic ultrasound, and carotid doppler were obtained. T-test, chi-square test, and simple logistic regression were done using STATA v12.

Results: Forty-six patients (mean age: 29.24 ± 8.63 years) were included in this study. Ten (21.7 %) had NAFLD based on ultrasound. Waist-to-hip ratio was higher (p -value < 0.011) in patients with NAFLD. Triglyceride levels were higher (p -value < 0.045) in NAFLD patients. Most NAFLD patients had hepatomegaly (90 %, p -value < 0.033) on ultrasound. Transaminases and GGT were not statistically different between the groups. Glucose Disposal Rate (eGDR) was significantly lower (p -value < 0.018) in the NAFLD group, signifying insulin resistance. Using logistic regression, there is no significant association between NAFLD and CVD (OR 4.25, CI 0.52–34.91, p -value < 0.178).

Conclusion: The results of this study suggest an absence of association between NAFLD and CVD. This may be attributed to the young population with less cardiovascular complications in our institution, compared to the population in other countries. Larger multicenter prospective studies are needed to further evaluate the association of NAFLD and CVD in type 1 diabetics.

P-0925

Association between caffeine consumption and nonalcoholic fatty liver disease in metabolic syndrome

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Background: Coffee consumption has an inverse association with the risk of cirrhosis and hepatocarcinoma in both alcoholic and non-alcoholic fatty liver disease (NAFLD). Some ingredient in coffee including caffeine may protect against cirrhosis. NAFLD was closely associated with components of metabolic syndrome. However, the correlation between caffeine consumption and NAFLD among this population is limited. This study aimed to evaluate the association between NAFLD and amount of caffeine consumption including calories intake in metabolic syndrome patients.

Methods: All metabolic syndrome patients according to the Harmonizing criteria were enrolled between November 2011 and January 2013 at Siriraj hospital. NAFLD was diagnosed by ultrasonography. Caffeine consumption was calculated from all drinks in milligrams per week (mg/week).

Results: A total of 505 metabolic syndrome patients had a mean age of 61.1 ± 10.9 years and 54 % were female. Mean body mass index was 27.1 ± 4.5 kg/m². The prevalence of NAFLD was 67.5 %. The mean of total caffeine consumption were 427 ± 432 and 493 ± 487 mg/week in the patients with and without NAFLD, respectively ($p = 0.238$). And the mean of total calories intake from drinks were 533 ± 595 and 564 ± 727 kilocalories per week, respectively ($p = 0.670$). The patients with NAFLD consumed more caffeine in tea (68 ± 157 vs. 60 ± 133 mg/week, $p = 0.006$) whereas the patients without NAFLD trended to intake more caffeine in coffee (385 ± 453 vs. 311 ± 341 mg/week, $p = 0.254$). Conclusions: A possible opposite role of caffeine with the presence of NAFLD in metabolic syndrome patients for caffeine consumption from coffee but it has different effect with caffeine intake from tea.

P-0926

Upregulated intestinal absorption of dietary saturated fatty acids in non-alcoholic steatohepatitis

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Background: Saturated fatty acids (SFAs) such as palmitic acid are an important risk factor for the onset and pathogenesis of non-alcoholic steatohepatitis (NASH). We aimed to clarify the changes of dietary SFAs intestinal absorption in NASH.

Methods: Participants were 33 healthy subjects (control) and 73 patients with NASH (32 with Brunt stage 1-2 (early NASH: e-NASH) and 41 with Brunt stage 3-4 (advanced NASH: a-NASH)). ¹³C-labeled palmitate (an SFA) was administered directly into the duodenum using gastrointestinal endoscopy to avoid delays resulting from delivery by stomach. Breath ¹³CCO₂ levels were then measured to quantify metabolized SFA. Apolipoprotein B-48 concentrations in postprandial serum after test meal were measured to quantify absorbed chylomicrons in the intestine. Expression of SFA transporters in intestinal specimens from these groups was also examined.

Results: Overall, ¹³CCO₂ excretion was significantly higher in the e-NASH group than in the control group ($p < 0.01$). Serum concentrations of apolipoprotein B-48 were higher in the e-NASH group than in the control and a-NASH groups. Moreover, mRNA and protein levels of cluster of differentiation 36 (CD36) and microsomal triglyceride transfer proteins (MTTP), which are molecules associated

with SFA uptake and chylomicron formation, were significantly higher in the e-NASH group ($p < 0.05$).

Conclusion: Significantly upregulated absorption of dietary SFA was evident in early NASH patients. This alteration in absorption of SFA would be associated with the increase of MTTP and CD36 in the jejunum. Those change of SFA metabolism might relate to the onset or pathogenesis of the early stages of NASH.

P-0927

The role of nutritions to incidence and severity of non alcoholic fatty liver disease

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Aim: To confirm the role nutrition on incidence and severity of NAFLD.

Methods: A Case control study, NAFLD were confirmed by ultrasonography and severity by NAFLD fibrosis score (low: < -1.5 , Intermediate: $-1.5-0.67$, High: > 0.67) and Carbohydrate, fat intake by Food Frequency Questionnaire, visceral fat deposition by Body Impedance Analysis.

Results: Thirty tree cases of NAFLD and 34 healthy control. The independent risk factors of NAFLD were hypertriglyceridemia (OR 8.7, 95 % CI 2.20-34.44) and deposition of visceral fat (OR 5.8, 95 % CI 1.39-24.82). Risk factors severity of NAFLD were carbohydrate and fat intake, visceral fat, metabolic syndrome and central obesity. The independent risk factor severity of NAFLD was only fat intake (OR 48.4, CI 95 % 2.78-844.1).

Conclusion: Deposition of visceral fat and hypertriglyceridemia were independent risk factors incidence of NAFLD and fat intake was independent risk factor severity of NAFLD.

Keywords: Visceral fat, hypertriglyceridemia, fat intake, severity, NAFLD

P-0928

Profile of patients diagnosed with fatty liver disease at Seamen's Hospital

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Fatty liver disease is a disorder characterize by fat accumulation in the hepatocytes which might lead eventually to cirrhosis and hepatocellular carcinoma. Seafarers are distinctive group of population working onboard afar from their family which makes them predispose to develop unhealthy lifestyle such as alcoholic intake. And this unhealthy lifestyle is the risk factor which predisposes the seafarer to develop different cardiometabolic diseases, and among them is the fatty liver disease. The data collected from this study will be able to determine the prevalence of fatty liver disease and the sociodemographic and clinical profile among seafarer seen at out-patient clinics of Seamen's hospital. The research utilizes cross sectional study using chart review of the seafarers diagnosed with fatty liver disease at outpatient clinics from 2007 to 2012 at Seamen's hospital. The data

collection was based on chart review utilizing a standard check list. The study reviewed a total of 184 charts with fatty liver disease among them 148 charts was found positive for fatty liver disease, with incidence of 80.4 %. Sociodemographically, the fatty liver of subjects were commonly seen among those who are in their 4th decade of life, those who works as officers, cooks and service crew onboard. Life-style factors reviewed noted that fatty liver disease is more related to those who does not smoke and to those who drinks less than 20 g of alcohol per day. Clinically, most metabolic diseases were related to fatty liver disease with higher incidence among patients with hypercholesterolemia followed by hypertension.

P-0929

The association of non-alcoholic fatty liver disease with metabolic syndrome

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Background: Non-alcoholic fatty liver disease (NAFLD) has been reported associated with features of metabolic syndrome. The aim of our study is focus on the strength of association between NAFLD with metabolic syndrome in a Chinese population.

Methods: Data from subjects who visited the Medical Screening Center at Taichung Verteran General Hospital were retrospectively collected from 2006 to 2009. The exclusion criteria included significant consumption of alcohol, chronic hepatitis B and C. These patients were assigned to two groups according to ultrasound findings: normal group and fatty liver group. Metabolic syndrome was diagnosed based on the 2005 International Diabetes Federation (IDF) criteria.

Results: Among all 7568 enrolled subjects, 5736 (75.8 %) and 1832 (24.3 %) patients were belong to the groups of normal and fatty liver, respectively. The individuals in the fatty liver group had significant male predominant (69.7 % vs. 56 %) and higher BWI (mean, 26.67 vs. 23.55 kg/m²) than those in the normal group. There were 441 (7.7 %) and 377 (20.6 %) cases having metabolic syndrome in the groups of normal and fatty liver respectively with significant difference. The most power strength of individual components of metabolic syndrome to NAFLD was hyperlipidemia (adjusted OR 4.49, 95 % CI 3.15 to 6.40) and hyperglycemia (adjusted OR 2.74, 95 % CI 2.15 to 3.49).

Conclusion: The individuals with NAFLD had a higher ratio of metabolic syndrome. Hyperlipidemia and hyperglycemia had the most power strength associations with NAFLD.

P-0930

The association of non-alcoholic fatty liver disease with obesity and apolipoproteins

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Introduction: Insulin resistance has been considered as the most important key mechanism in development of non-alcoholic fatty liver

disease (NAFLD). Some research studies reported that hyperinsulinemia decreases the hepatic secretion of Apo B. Chronic hyperinsulinemia in NAFLD may be responsible for accumulation of triglycerides within hepatocytes.

Aim: We aimed to investigate whether there is a relationship between apolipoproteins and histological findings in patients with biopsy-proven NAFLD. We also aimed to evaluate the effect of obesity on apolipoproteins and pathogenesis of NAFLD.

Methods: A total of 91 patients with biopsy-proven NAFLD were included. The control group consisted of 39 healthy subjects who have no history of liver disease and alcohol consumption, matched for age, sex and smoking. Apolipoprotein A1 and Apolipoprotein B were measured by immunoturbidimetric method with commercially available OSR6142 Apolipoprotein A1 and OSR6143 Apolipoprotein B immunoassay kits using the Olympus AU2700 analyzer.

Results: Age, sex, and smoking distribution were similar among non-alcoholic steatohepatitis, simple steatosis, and control. The difference among NASH, SS, and control subjects in mean Apo A1, Apo B levels, and Apo B/A1 ratio did not reach statistical significance subjects ($p > 0.05$, for all comparisons). In addition, patients with obese NAFLD had higher score of steatosis than patients with non-obese NAFLD ($p < 0.05$).

Conclusion: The present study showed that Apo A1, B, and B/A1 ratio are not associated with histopathological findings in patients with NAFLD. In addition, obesity only increases the grade of hepatic steatosis but does not cause lobular inflammation, ballooning and fibrosis.

P-0931

The risk of preclinical atherosclerosis in non-diabetic, non-hypertensive adults by NAFLD

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Objectives: Nonalcoholic fatty liver disease (NAFLD) is associated with CVD, yet whether identification of NAFLD in non-diabetic, non-hypertensive adults is helpful over other established risk factors remains unknown.

Methods: A total of 24,377 non-diabetic, non-hypertensive adults aged 20 years or older who underwent comprehensive health check-up including carotid and abdominal ultrasonography was analyzed. NAFLD was diagnosed with ultrasonography and exclusion of secondary causes of fat accumulation or other causes of chronic liver disease. Metabolic syndrome (MetS) was defined by the Adult Treatment Panel III criteria. Preclinical atherosclerosis was defined when carotid plaque was present.

Results: NAFLD was independent factor associated with carotid plaque [adjusted odds ratio (OR) (95 % confidence interval (CI)): 1.18 (1.10–1.25)]. MetS was specific (0.90), but not sensitive (0.12) for carotid plaque. NAFLD was more sensitive (0.39) than MetS, but less specific (0.65). There were 9,245 metabolic healthy participants, defined by no metabolic syndrome component, yet, carotid plaque was present in 30.1 % of participant. NAFLD was independent factor associated with carotid plaque [adjusted OR (95 % CI): 1.25 (1.11–1.42)] in metabolic healthy participants, and was specific (0.84) for carotid plaque in this population, but sensitivity was low (0.17).

Conclusions: In non-diabetic, non-hypertensive adults, NAFLD was independent factor for carotid plaque, and could identify additional patients at risk for CVD with reasonable specificity, especially among

metabolic healthy population. This finding suggests NAFLD can be early surrogate for CVD that can be used to reduce CVD risk before establishment of MetS.

P-0932

PPAR α can attenuate dietary trans-fatty acid-induced liver changes

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Trans-fatty acids (TFA) are defined as unsaturated fatty acids (FA) containing trans double bonds and its consumption is considered as a risk factor for metabolic syndrome. Some studies show that excess TFA intake is susceptible for hepatic steatosis and inflammation. While peroxisome proliferator-activated receptor α (PPAR α) is known to be associated with hepatic lipid metabolism and inflammatory pathways, the role of PPAR α for TFA-induced liver changes remains undetermined. In this study, wild-type and Ppara-null mice were treated with a control diet or isocaloric one that replaced all FA to TFA by hydrogenation and liver phenotypes were examined after 2 months. Ppar α -null mice fed a TFA-replacing diet showed significant increases in hepatic triglyceride (TG) contents and serum alanine aminotransferase levels and severe steatosis and inflammation compared with similarly-treated wild-type mice. While TFA intake increased hepatic mRNAs encoding enzymes associated with FA synthesis and TG hydrolysis in both genotypes, more severe steatosis in Ppara-null mice was mainly derived from reduced FA utilization/degradation. Additionally, the mRNA levels of genes related to inflammation and fibrosis, such as osteopontin and collagen 1 α 1, tended to be increased and nuclear factor kappa B was activated in TFA-fed Ppar α -null mice. Enhanced inflammatory signaling in these mice was presumably mediated by Toll-like receptor 2, but not inflammasome activation. Collectively, these results indicate that PPAR α plays a protective role for dietary TFA-induced liver abnormalities.

P-0933

Relationship between patient lifestyle and development of fatty liver disease

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Introduction: Way of lifestyle plays a significant role in the origins of fatty liver disease. Therefore, it is so important to identify and reduce the lifestyle risk factors contributing to the development of fatty hepatosis.

Materials and methods: retrospective–prospective study included 105 patients from Infectology Center of Latvia Hepatology department. Study group (SG) includes 75 patients with morphologically

proven fatty liver disease. Control group (CG) includes 30 patients with chronic hepatitis C without steatosis or cirrhosis. Patients from both groups were interviewed about their lifestyle and health conditions with self-developed questionnaire.

Results: SG respondents with fatty liver disease have been statistically significantly older (47.64 ± 11.04) than respondents from CG (40.87 ± 11.29) ($p = 0.006$), however, a statistically significant difference between genders was not observed. Also a larger BMI was observed, in SG $29.92 \text{ kg/m}^2 \pm 6.01$, CG $24.90 \text{ kg/m}^2 \pm 3.26$ ($p = 0.001$). Type II diabetes and hypercholesterinaemia prevails more often in SG respondents than in CG ($p < 0.05$). Occurrence of increased physical activity in SG has been 3–1 times a week. SG respondents use more often – 70.0 % ($n = 40$) easily assimilable carbohydrates than CG – 40.0 % ($n = 21$), ($p < 0.05$), more than a couple of times a week, as well as they use more often products high in saturated fat—70.0 % ($n = 53$) against 50.0 % ($n = 15$), $p < 0.005$.

Conclusion: Lifestyle factors, such as using easily assimilable carbohydrates and foods highest in saturated fat, low level of physical activity (<3 times a week), as well as type II diabetes and hypercholesterinaemia, are statistically significant risk factors for development of fatty liver disease.

P-0934

Is serum uric acid risk factor for non-alcoholic fatty liver disease?

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Background: The association between elevated serum uric acid (SUA), individual components of the metabolic syndrome and Non-alcoholic fatty liver disease (NAFLD) was demonstrated in the several clinical studies. There are lots of conflicting results of association between SUA and NAFLD. The aim of the present study was to evaluate the relationship of SUA with liver histopathology in patients with NAFLD.

Patients and methods: In this retrospective study, a total of 362 patients (NASH: 220, simple steatosis (SS): 142) with biopsy proven NAFLD were included. Kleiner's scoring scale was used for histopathological examination. Demographic characteristics, liver function tests, insulin were documented. Enzymatic colorimetric method with an Olympus AU2700 (Beckman Coulter, USA) auto analyzer was used to detect SUA levels. Hyperuricemia was considered when the SUA rises above 7 mg/dL.

Results: The mean age of patients were 40.03 ± 8.7 years. Sixty (16.6 %) patients were women. The prevalence of hyperuricemia was 32.9 % in patients with NAFLD. No significant difference in SUA levels was found between NASH and SS groups ($P = 0.202$). In correlation analysis, we did not show significant association between SUA levels and histopathological findings in patients with NAFLD ($r = 0.032$, $P = 0.547$). In addition, there was no difference between NAFLD with fibrosis and without fibrosis in terms of SUA levels ($P > 0.05$).

Conclusions: We demonstrated that high prevalence of SUA in NAFLD patients when compare to the general population. Our study showed that SUA in patients with NAFLD are not associated with histopathological findings such as NAS and fibrosis.

P-0935

Limited implication of ratio of serum AST to serum ALT for diagnosis of NASH

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Aim: Studies have shown that ratio of serum aspartate aminotransferases (AST) to serum alanine aminotransferases (ALT) may have implication in the diagnosis of non-alcoholic steatohepatitis (NASH), however, there is lack of consensus about this issue.

Methods: An observational, cross sectional study was carried out in the Department of Hepatology, Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh. Forty three patients with NAFLD were enrolled in this study. Diagnosis of NAFLD was made at abdominal ultrasonography and all patients subsequently underwent per-cutaneous liver biopsies for NAS scoring to distinguish NASH from non-NASH fatty liver. AST to ALT ratio of all 43 patients were analyzed.

Results: Twenty patients had NASH and non-NASH NAFLD was diagnosed in 23 patients. AST to ALT ratio ≤ 1 was seen in 75.0 % NASH patients and 82.6 % non-NASH NAFLD patients. AST to ALT ratio did not significantly correlate ($p = 0.54$, chi-square test) to distinguish NASH from non-NASH fatty liver.

Conclusion: Serum AST to ALT ratio did not significantly predict NASH. Biopsy is still the gold standard to distinguish steatohepatitis and/or fibrosis.

P-0936

Measurement of liver fat volume by multi-material decomposition method with dual energy CT in NAFLD

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Background and aim: Non-alcoholic fatty liver disease (NAFLD) may cause steatohepatitis resulting in cirrhosis over time and may even lead to hepatocellular carcinoma or liver failure. In this study, we have developed a new noninvasive method for quantitative measurement of fat volume of the liver by multi-material decomposition (MMD) method with dual energy CT.

Methods: 44 NAFLD patients with histological diagnosis were enrolled. By MMD method with dual energy CT, Discovery 750HD(GE Healthcare) that can create Gemstone Spectral Imaging, we measured liver fat volume and compared with NASH activity score (NAS) fat score and liver to spleen attenuation ratio. We also calculated the proportion of fat area in liver biopsy specimens and compared it with liver fat volume measured by MMD method with dual energy CT.

Results: Liver fat volume measured by MMD method significantly correlated with liver to spleen attenuation ratio ($P < 0.001$, $r = -0.851$). The median of liver fat volume of patients with 0 to 3 NAS fat score measured by MMD method was 1.88 % (range 0.56–9.3; $n = 9$), 10.4 % (1.52–17.8; $n = 19$), 12.7 % (2.77–23.8; $n = 14$), and 15.3 % (8.52–22.1; $n = 2$), respectively, and positive correlation was observed between NAS fat score and liver fat volume measured by MMD method ($P < 0.001$). Also, there was a positive correlation between liver fat volume measured by biopsy specimen and by MMD method ($P < 0.001$, $r = 0.707$).

Conclusion: Measurement of fat volume of the liver by MMD method with dual energy CT can be a useful tool for the diagnosis of NAFLD.

P-0937

Relationship between Vitamin B12 levels and hepatic fibrosis detected with fibroscan in NAFLD

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In our study, we tried to reveal the possible relation between staging of steatosis and fibrosis determined by fibroscan technique and serum vitamin B12 levels as a biomarker in NAFLD patients.

Methods: A total of 129 patients (45.68 ± 12.9 years and 29F) with NAFLD and 50 healthy subjects (43.44 ± 15.3 years and 21F) were included in this study. Fibroscan is performed in all patients for staging of the fatty liver fibrosis. Liver enzymes are also analyzed in addition to serum vitamin B12 and CRP levels. NAFLD patients were divided into five groups according to fibroscan scores from F0 to F4. To compare the parameters between test and control groups, independent t-test was used.

Results: There was no difference in terms of age and gender in NAFLD and control groups. The serum ALT, AST, GGT and CRP levels were significantly higher in the NAFLD patients than controls ($p < 0.05$). On the contrary, serum vitamin B12 vitamin levels were lower in patients with NAFLD compared with controls (352.8 ± 124 pg/mL vs. 435.2 ± 134 , $p < 0.01$). There was a significant difference in mean serum B12 vitamin levels between controls (435.2 ± 134 pg/mL) and NAFLD patients from F0 to F3 subgroups ($366,17 \pm 129.7$ pg/mL, 285.22 ± 101 pg/mL, $p < 0.01$).

Conclusion: The serum vitamin B12 levels were found to be significantly low in patients with NAFLD when compared to the control group. The reduction in serum vitamin B12 levels was even more

pronounced as hepatic inflammation and fibrosis increases but not in advanced fibrosis stage.

P-0938

Role of paraoxonase-1, caspase cleaved cytokeratin 18 and pentraxin-3 levels in NAFLD

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Aim: In this study we compared the diagnostic accuracy of serum levels paraoxonase-1 (PON-1), pentraxin-3 (PTX-3) and caspase cleaved cytokeratin 18 (CTK-18) in determining improved stages of disease in Non alcoholic fatty liver disease (NAFLD) and by this way we aimed to support the studies for developing non-invasive methods. **Material and Method:** Forty four patients, 14 diabetic and 30 non-diabetic, diagnosed with non-alcoholic steatohepatitis (NASH) with liver biopsy, aged between 22 and 74 years and 42 healthy controls without any known systemic diseases, totally 86 cases, were included in the study. Waist circumference, body mass index, liver function tests, lipid profiles, fasting insulin levels (HOMA index), fasting blood glucose, serum caspase cleaved cytokeratin-18, paraoxonase-1 and pentraxin-3 levels were studied and compared with the findings of liver biopsy. The results are evaluated statistically.

Results: In NASH group, serum AST, ALT, GGT, TG, PTX-3 and caspase cleaved CTK-18 levels and HOMA indices were statistically significantly higher than healthy adults and serum PON-1 levels were significantly lower. Additionally, when patients were evaluated separately, in NASH group, with an increase in steatosis, ballooning degeneration and fibrosis stages, serum PTX-3 and caspase cleaved CTK-18 levels were also statistically significantly increasing.

Conclusion: According to these results, serum AST, ALT, GGT, TG, PTX-3, PON-1 and caspase cleaved CTK-18 levels may be used as the NASH marker in NAFLD. Additionally, serum PTX-3 and caspase cleaved CTK-18 levels may be used in determination of fibrosis stage in NASH.

P-0939

Serum PEDF as an independent biomarker in non-invasive diagnosis of NASH

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Background: Non-alcoholic Fatty Liver Diseases (NAFLD), especially its critical stage of non-alcoholic steatohepatitis (NASH), has become one of the most important public health issues worldwide. Non-invasive diagnosis has become the evitable trade of NASH diagnosis and treatment. In the study, we researched the correlation between PEDF and the progression of NAFLD and diagnosed NAFLD non-invasively.

Method: 136 patients with biopsy-proven NAFLD and 83 age- and gender-matched healthy subjects were enrolled. Height, weight, waist circumference and other physiological indicators were measured.

ALT, AST, AST/ALT, ALP, GGT, TC, TG, HDL, LDL and other biochemical markers were detected. Serum level of PEDF was measured by enzyme-linked immunosorbent assay.

Results: Serum level of PEDF in NASH patients was significantly higher than Non-NASH patients ($P < 0.01$) and PEDF can differentiate borderline NASH from other groups ($P < 0.05$). Serum level of PEDF was associated with ALT, AST, GGT, TC, TG and BMI ($P < 0.05$), and highly correlated with hepatic steatosis, ballooning degeneration, lobular inflammation, periportal inflammation and fibrosis degree ($P < 0.01$). Serum level of PEDF increased with NAS rating and indicated a high-positive correlation ($r = 0.835$, $P < 0.01$). ROC curve analysis area of PEDF was 0.886. Optimum sensitivity, specificity and the best cut-off value were determined by Youden index as 79.6, 78.4 % and 23.96 $\mu\text{g/L}$ respectively, which revealed high diagnostic value of PEDF.

Conclusion: Serum level of PEDF was significantly increased in NASH, which could be an independent predictor to evaluate NASH and a non-invasive diagnostic indicator for NASH.

Keywords: PEDF; NASH; Non-invasive diagnostic

P-0940

Serum 25(OH)D3 levels are related to the severity of liver fibrosis in patients with NAFLD

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Background: Previous studies suggested that serum vitamin D levels in patients with non-alcoholic fatty liver disease (NAFLD) are lower than in those with healthy subject. However, the influencing of vitamin D deficiency in Japanese patients with NAFLD remains unclear. Therefore, this study aimed to clarify the relationship between the pathogenesis in patients with NAFLD and serum vitamin D levels in Japan.

Methods: We investigated serum 25(OH)D3 levels of 112 patients who were defined as NAFLD by liver biopsy. As control subjects, we also measured serum 25(OH)D3 levels of 80 healthy volunteers. The study was approved by the Ethics Committee of Nippon Medical School Chiba Hokusoh Hospital.

Results: Serum 25(OH)D3 levels were significantly lower in patients with NAFLD (20 ng/mL, range 6–40) than in healthy subjects (25 ng/mL, range 7–52) ($P = 0.005$). Serum 25(OH)D3 levels were significantly lower in patients with advanced fibrosis (stages 3–4) than in those with mild fibrosis ($P = 0.013$). There were significantly negative correlations between serum 25(OH)D3 levels and various fibrosis markers such as hyaluronic acid, but no correlation was found between activity scores of NAFLD and serum 25(OH)D3 levels. In multivariate analysis, platelets ($P = 0.003$, OR = 0.864, 95 % CI = 0.785–0.951), fasting plasma glucose ($P = 0.043$, OR = 1.015, 95 % CI = 1.001–1.029) and serum 25(OH)D3 level ($P = 0.013$, OR = 0.907, 95 % CI = 0.840–0.979) were identified independent factors contributing advanced fibrosis.

Conclusions: In this study, serum 25(OH)D3 levels were related to the severity of fibrosis in patients with NAFLD in Japan.

P-0941

Serum Apelin as a non-invasive marker for nonalcoholic steatohepatitis**Gasser El-Azab¹, Mohamed Abdelgawad², Gamal Abo-Raia³, Eman Abdelzaher⁴, Rania Abouyoussef⁵, Ehab Elkhoully⁵**

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Background: Nonalcoholic fatty liver disease (NAFLD) consists of a spectrum of conditions including simple steatosis, steatohepatitis (NASH), fibrosis and cirrhosis. It is associated with the metabolic syndrome and insulin resistance (IR). Apelin is the endogenous ligand of APJ, which belongs to the family of G protein-coupled receptors. It is an adipokine that was recently suggested to be associated with IR, Obesity, inflammation and fibrosis progression. We evaluated serum apelin as a noninvasive maker for discriminating NASH from simple steatosis, and its correlation with the disease severity.

Methods: Serum apelin levels were measured in 140 patients with histologically proven NAFLD (88 had steatosis and 52 NASH) and 40 healthy control subjects. HOMA-IR was calculated and visceral fat (VF) was measured by ultrasound.

Results: Serum apelin level was significantly higher in the NASH cases than in steatosis cases and control subjects ($P < 0.001$). Apelin levels were positively correlated with the stages of liver fibrosis, NAFLD activity scores, HOMA-IR and VF. These results were consistent when apelin was adjusted for confounders. For detection of NASH, the area under the curve (AUC) was 0.797 ($P < 0.001$) by ROC analysis; the best cutoff value of serum apelin was 2.225 ng/ml. AUC to distinguish significant fibrosis (F3–F4) was 0.934 ($P < 0.001$) with cutoff value of 2.94 ng/ml. Conclusions: Serum apelin measurement is novel, noninvasive tool to differentiate NASH from steatosis, and to assess the severity of liver fibrosis in NAFLD patients. It is correlated with IR and adiposity, and may become a potential therapeutic target of NASH treatment.

P-0942

The association between sAIM and the clinical features of non-obese patients with NAFLD**Kohei Oda¹, Hirofumi Uto², Yoshio Sumida^{3,4}, Takeshi Okanoue⁵, Seiichi Mawatari¹, Rie Ibusuki¹, Sho Ijyuin¹, Hiroka Onishi¹, Haruka Sakae¹, Kaori Muromachi¹, Eriko Tabu¹, Akihiko Oshige¹, Tsutomu Tamai¹, Akihiro Moriuchi⁶, Hirohito Tsubouchi^{6,7}, Akio Ido^{1,6}**

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Background and aims: NAFLD is a condition associated with metabolic syndrome and insulin resistance. We also, however, see cases in daily practice of NAFLD in individuals with normal BMI. Apoptosis inhibitor of macrophage (AIM) is a protein specifically produced by macrophages that is reported to be involved in metabolic syndrome and insulin resistance. We previously reported that AIM contributes to the progression of pathology in NAFLD. The aim of this study was to elucidate the role of AIM in non-obese patients with NAFLD.

Methods: We analyzed 257 patients with biopsy-proven NAFLD, including 205 with NASH and 52 with NAFL, and investigated the association between serum AIM (sAIM) levels and clinical features.

Results: (1) The non-obese group (BMI < 25 , 67 patients) was older, and had less visceral fat and subcutaneous fat and less insulin resistance than the obese group (BMI ≤ 25 , 190 patients). There were no significant differences in sAIM. (2) In the non-obese group, AST, type IV collagen 7S and HOMA-IR were higher in patients with NASH (51 patients) than in those with NAFL (16 patients). sAIM were significantly higher in the NASH group than in the NAFL group. sAIM were significantly correlated with markers of hepatic fibrosis, and were associated with histopathological findings in the liver. Furthermore, there were significant negative correlations between sAIM and liver fat content.

Conclusions: sAIM were high in both obese and non-obese patients with NAFLD, and AIM has been associated from NAFLD in the pathology progress of NASH.

P-0943

The relationship of pentraxin 3 with atherosclerosis in non-alcoholic fatty liver disease**Omer Kurt¹, Kadir Ozturk¹, Tolga Dogan², Alptug Ozen³, Fatih Yesildai⁴, Hakan Demirci¹, Ahmet Uygun¹, Sait Bagci¹**

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Objective: Several clinical studies have focused on finding a biomarker which is most closely correlated with the severity of liver fibrosis in NAFLD, but already failed to prove a serum biomarker for accurately predicting the severity of liver fibrosis. The aim of the present study was to investigate whether pentraxin 3 (PTX3) can be a new non-invasive marker for prediction of liver fibrosis in NAFLD. We also aimed to evaluate the relationship between PTX3 and atherosclerosis in NAFLD.

Method: Fifty-four male patients with biopsy-proven NAFLD who have no CVD risk factors and 20 apparently healthy male volunteers were included in the study. PTX3 levels were determined, using an ELISA method (R&D Systems, Quantikine ELISA, USA). To detect the presence of subclinical atherosclerosis in patients with NAFLD, measurements of CIMT, FMD, and cf-PWV levels in all participants were performed.

Results: PTX3 levels in NAFLD patients with fibrosis were higher than both NAFLD patients without fibrosis and controls ($P = 0.032$ and $P = 0.028$, respectively), but there was no different between controls and NAFLD patients without fibrosis in terms of PTX3 levels ($P = 0.903$). PTX3 levels were strongly correlated with cf-PWV ($r = 0.359$, $P = 0.003$), whereas no significant correlation was found with other atherosclerosis markers, CIMT and FMD.

Conclusion: Elevated plasma PTX3 levels are associated with the presence of fibrosis in patients with NAFLD, independently of metabolic syndrome components. NAFLD patients with fibrosis have increased risk of atherosclerosis. There is a close association between elevated PTX3 levels and increased arterial stiffness in patients with NAFLD.

P-0944

The usefulness of markers for diagnosis of NAFLD comparing with chronic hepatitis C

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Aim: Serum high molecular weight adiponectin (HMWA), leptin, TNF-alpha and CK18 have been proposed as markers for nonalcoholic fatty liver disease (NAFLD). The aim of this study was to elucidate the usefulness of these markers in NAFLD by comparing between NAFLD and chronic hepatitis C (CHC).

Methods: 85 NAFLD patients and 52 CHC patients were evaluated. Velocity of shear wave (Vs) correlated with liver fibrosis was measured by ARFI.

Results: Gender and age (years) did not differ between NAFLD and CHC (33/52 vs. 23/29; 58 vs. 57). BMI and ALT (IU/L) was significantly higher in NAFLD than in CHC (27 vs. 23, $p < 0.0001$, 76 vs. 46, $p = 0.0033$). Vs (m/s) tended to be higher in NAFLD than in CHC (1.9 vs. 1.6, $p = 0.0546$). HMWA ($\mu\text{g/mL}$) was significantly higher in CHC than NAFLD (5.0 vs. 3.6, $p = 0.0260$). Leptin (ng/mL) and CK18 (U/L) were significantly higher in NAFLD than in CHC (14 vs. 8, $p = 0.0060$; 311 vs. 211, $p = 0.0071$). TNF-alpha did not differ between two diseases. Vs was significantly or in tendency correlated with HMWA ($r = 0.258$, $p = 0.0865$), TNF-alpha ($r = 0.289$, $p = 0.0512$), leptin ($r = 0.321$, $p = 0.0299$) and CK18 ($r = 0.564$, $p < 0.0001$) in NAFLD. Vs was significantly or in tendency correlated with HMWA ($r = 0.262$, $p = 0.0690$), TNF-alpha ($r = 0.381$, $p = 0.0069$) and CK18 ($r = 0.304$, $p = 0.0336$) in CHC.

Conclusion: Although ALT was significantly higher in CHC than in NAFLD, leptin and CK18 were significantly higher in NAFLD than in CHC, and were significantly correlated with Vs in NAFLD. These findings suggest that leptin and CK18 are useful for diagnosis of NAFLD.

P-0945

Use of biochemical and metabolic markers for the detection of nonalcoholic steatohepatitis (NASH)

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Background: Obesity is one of the main risk factors for development of NASH. The gold standard for the diagnosis of NASH is liver biopsy; however, it is a procedure difficult to perform in high risk populations.

Aim: To analyze if AST > 54 UI/L, ALT > 42 UI/mL, glucose > 100 mg/dl, triglycerides (TG) > 150 mg/dl and HOMA > 2.5 as biochemical and metabolic markers (BMM), can improve the detection of NASH.

Methods A cross-sectional study was performed in 383 subjects classified as Group 1 ($n = 318$, weight range: normal to obesity class 2) and Group 2 ($n = 65$ candidates for bariatric surgery). Anthropometrics was assessed by bioelectrical impedance (Inbody). BMM were evaluated by a dry chemistry assay. Patients with one or more altered BMM were screened by transitional elastography (TE) (Fibroscan™) in Group 1, whereas liver biopsy was performed in Group 2.

Results: In Group 1, 57 % (181/318) presented 1 or > 1 BMM abnormalities. Among these, 102 altered-BMM patients were screened by TE of which 53 % (54/102) had F1 to F4 liver stiffness. In Group 2, 87 % (57/65) had 1 or > 1 altered BMM and 91 % (52/57) had fibrosis or cirrhosis. Overall, the OR of association of the BMM with NASH was 8.18 (95 % CI 3.21–20.87, $p < 0.001$); whereas hypertriglyceridemia and HOMA showed an OR = 1.21 (95 % CI 1.03–1.28) and OR = 4.38 (95 % CI 2.97–6.45) ($p < 0.001$), respectively.

Conclusions Use of BMM allowed an enhanced detection of NASH among patients with normal weight up to obesity class 3. The prevalence of NASH in each population may change by using this present strategy.

P-0946

Changes of alanine aminotransferase and gamma glutamyl transpeptidase with histology of fatty liver

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Background: Study on dynamic changes of nonalcoholic fatty liver disease activity score (NAS) and alanine aminotransferase (ALT) and gammaglutamyl transpeptidase (GGT) is scarce. This study is to explore the dynamic changes of ALT and GGT with changes of NAS and fibrosis.

Methods: Nonalcoholic fatty liver disease (NAFLD) patient of 678 was included after exclusion of other causes of hepatitis and fatty liver. Liver biopsy was done once in 263 cases with metabolic syndrome. Paired liver biopsy done at 1 year interval after life style modification and pentoxifylline/Telmisartan therapy in 61 cases of nonalcoholic steatohepatitis (NASH). Liver histology was scored as NAS.

Results: In NAFLD cases age was (40.2 ± 9.8) years, male: female was (268:410). ALT, AST and GGT was 57.2 ± 48.6 , 43.4 ± 41.8 and 51.1 ± 35.7 μl respectively. Of them 347(51.2 %) had normal ALT. ALT and GGT was positively correlated with NAS ($p < 0.01$) and fibrosis ($p < 0.05$). ALT ($p < 0.005$) and GGT ($p < 0.0001$) was significantly higher in NASH than that of non NASH. ALT > 40 μl could detect NASH with sensitivity and specificity of 69.4 and 49.5 %, GGT > 38 μl with sensitivity and specificity of 64.9 and 56.2 %. Changes of NAS after 1 year was positively correlated with GGT ($p < 0.01$) but not with ALT ($p < 0.726$). Changes of fibrosis score was neither correlated with ALT nor GGT.

Conclusion: Half of the NAFLD patient had normal ALT. ALT and GGT could detect NASH with moderate sensitivity and specificity. Dynamic changes in NAS could be reflected with GGT but not with ALT.

P-0947

Controlled attenuation parameters is efficient in the quantification of hepatic steatosis

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Aim: The current gold standard for the diagnosis and staging of hepatic steatosis is liver biopsy. A novel, non-invasive technology that is easy and quickly done, Controlled Attenuation Parameters (CAP), has recently become available for clinical use. The aim of this cohort study was to evaluate the efficiency of CAP measurement in the quantification of hepatic steatosis.

Methods: A total of 151 Japanese patients with chronic liver disease had CAP values determined at the same time as liver biopsy to examine the relation between the CAP value and hepatic histological findings. The stage of steatosis (S0-3) was established according to the NAFLD activity score (S0, steatosis in <5 % of hepatocytes; S1, 5-33 %; S2, 34-66 %; S3, >66 %). The relation between elevation of the CAP value and clinical factors was also investigated.

Results: The CAP value was significantly correlated with the steatosis grade ($P < 0.001$, $\rho = 0.508$). CAP had reasonable AUROC values of 0.782 for $\geq S1$ (95 % CI, 0.693-0.851) and 0.844 for $\geq S2$ (95 % CI, 0.658-0.938). The optimal CAP value cut-offs for the determination of steatosis grade were 218 dB/m for $\geq S1$ (sensitivity 79.0 % and specificity 73.0 %) and 251 dB/m for $\geq S2$ (sensitivity, 85.7 % and specificity, 82.5 %). In multivariate analysis, BMI > 25 kg/m² and γ -GTP > 50 IU/L were associated with an elevation of CAP value of more than 251 dB/m.

Conclusion: CAP measurement is efficient in the quantification of hepatic steatosis, making it clinically useful for screening, staging, and monitoring.

P-0948

Relation of risk factors between metabolic syndrome and NAFLD in apparently healthy young adults

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Background and aims: The prevalence of non-alcoholic fatty liver disease (NAFLD) has increased and several studies have shown that there is an association between NAFLD and metabolic syndrome (MetS). The aim of this study was to determine how much impact the risk factors of MetS has on ultrasonographic fatty liver, especially NAFLD in young people (20 to 40 years old).

Methods: This cross-sectional study enrolled 2,648 adults (1422 males and 1226 females) undergoing a general health checkup from May 2012 to April 2014. An ultrasonographic evaluation of abdomen

was conducted to evaluate NAFLD. The diagnosis of the MetS was assessed as defined by the International Diabetes Federation consensus while abdominal obesity was assessed according to Asia-Pacific guidelines. Factors related to MetS were assessed by Chi square test and logistic regression analysis.

Results: MetS was diagnosed in 17.9 % with ultrasonography-diagnosed NAFLD. Body mass index, waist circumference, fasting blood glucose, triglyceride (TG), high-density lipoprotein cholesterol and aspartate aminotransferase, alanine aminotransferase, γ -glutamyl transpeptidase levels all affected NAFLD independently. Especially, TG was strongly correlated with MetS in NAFLD. The prevalence of MetS was increased in mild (29.6 %) and moderate (47.2 %) NAFLD groups. When odd ratio [95 % confidence interval (CI)] for NAFLD group was compared to the contrast group, there was an increased risk of MetS with odd ratio of 11.3 (95 % CI, 7.1~15.0).

Conclusions: NAFLD and its severity has a close connection with MetS and also with each risk factors of MetS. Therefore, assessment for concurrent MetS among NAFLD patients is considered to be necessary.

P-0949

Metabolic syndrome as predictors for severity of NAFLD detected by ultrasonography in Taiwanese

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The nationwide HBV vaccination successfully reduced the incidences of HCC and HBV infection in Taiwan, but the non-alcoholic fatty liver disease (NAFLD) impact on hepatocellular carcinoma (HCC) was thought highly respect for prevention recently, which associated with metabolic syndrome (MetS). However, NAFLD detecting by AUS must highly depend on professional clinical manpower for general population approach. Therefore, our study aim is to generate predictors for NAFLD, which based on the association between metabolic factors and NAFLD severity. Changhua County had conducted a community-based integrated screening (CHCIS) program provided HCC and other neoplastic diseases (breast, colorectal, oral, and cervical cancers) and chronic diseases (hyperlipidemia, hypertension, and hyperglycemia) screening which was launched since 2005. Following CHICS, AUS was designed and targeted at residents aged 45-69 years. The NAFLD severity was diagnosed into 4 levels (normal, mild, moderate, and severe) by clinical gastroenterology physicians. The biomarkers and questionnaire were implemented for personal information collection. The polytomous logistic regression was conducted to analyze the relationship between the NAFLD severity level and MetS components. Excluding the hepatitis and alcohol drinking, total 5810 subjects were recruited. The overall NAFLD prevalence is 39.80 %. After adjustment for age, gender, and smoking, compared with normal, the significant factors included each MetS components and elevated GPT, but inverse relationship with GOT, which showed in mild and moderate/severe, but striking association was demonstrated in moderate/severe. The high prevalence of NAFLD was revealed in general Taiwanese adults. Those MetS factors could successfully predict the severity of NAFLD for Taiwanese general population.

P-0950

Which is more important for development of endothelial dysfunction: steatosis or fibrosis?**Ferdane Sapmaz¹, Metin Uzman¹, Sebahat Basyigit¹, Selcuk Ozkan², Bunyamin Yavuz², Abdullah Yeniova¹, Ayse Kefeli¹, Zeliha Asilturk³, Yasar Nazligul¹**¹Kecioren Education and Training Hospital Gastroenterology Department, Ankara, Turkey; ²Kecioren Education and Training Hospital Cardiology Department, Ankara, Turkey; ³Kecioren Education and Training Hospital Internal Medicine Department, Ankara, Turkey**Background and aim:** It is shown that there are strong associations between Non-alcoholic fatty liver disease (NAFLD) and endothelial dysfunction. The aim of our study was to reveal whether steatosis or fibrosis score is more important in the development of endothelial dysfunction in patients with NAFLD in a prospective manner.**Material and method:** Overall, 266 cases were included to the study. The cases were assigned into 2 groups according to presence of hepatosteatosis based on sonography findings. Cases with hepatosteatosis were stratified according to severity of steatosis: grade 1, 2 and 3. In all patients, AST-to-Platelet Ratio Index (APRI) and Fibrosis-4 (FIB4) scores were calculated. In addition, flow mediated dilatation (FMD) measurements were recorded.**Findings:** There was NAFLD in 176 (66.2 %) of 266 cases included. There were no significant differences in sex and age distributions between patients with NAFLD (Group 1) and controls without NAFLD (Group 2) ($p = 0.05$). Mean APRI score was significantly higher in group 1 compared to controls ($p = 0.001$) while no significant difference was detected regarding FIB4 scores between groups ($p = 0.4$). Mean FMD value was found to be significantly lower in group 1 ($p = 0.008$). Patients with grade 3 hepatosteatosis had significantly lower FMD values than those with grade 1 steatosis and controls ($p = 0.001$). In univariate and multivariate analyses in group 1, no significant difference was detected regarding mean FMD measurements ($p = 0.03$). Again, no significant difference was detected in mean FMD measurement between FIB4 subgroups within patients with NAFLD and whole study group ($p = 0.09$).**Conclusion:** The endothelial dysfunction is associated to steatosis in patients with NAFLD.

P-0951

Possible gender difference of NAFLD in adolescence evaluated by elevated liver enzyme levels**Chiharu Yoshida¹, Mari Matsumoto¹, Eiko Suzuki¹, Setsuko Kikuchi¹, Yoshiaki Maruyama¹, Tomoaki Tomiya^{1,2}**¹Health Promotion Center, Saitama Medical University, Saitama, Japan; ²Department of Gastroenterology and Hepatology, Saitama Medical University, Saitama, Japan**Background and aim:** NAFLD is considered to have the potential to progress to liver cirrhosis in addition to forecasting an increased risk of diabetes and metabolic syndrome. Diagnosis of NAFLD early in life is important to prevent future liver- and metabolically-related serious outcomes in adulthood. We studied prevalence of NAFLD evaluated by elevated liver enzyme levels in relation to BMI and its gender difference in Japanese adolescence.**Methods:** A total of 387 medical students admitted to our university in last 3 years (M:F = 254:133, mean age: 20 y.o.) was enrolled. None of participants consumed excessive quantities of alcohol by self-reporting and were positive for HBsAg or HCVAb. We defined raised serum AST, ALT and gamma-GT levels as greater than 38, 30 and 50 IU/L, respectively.**Results:** BMI in 18 % of males and 2 % of females indicated as obese (BMI>25), while 51 % of males and 75 % of females showed BMI less than 22. Serum AST, ALT and gamma-GT levels were increased in 6.7/0 % (M/F), 31/3 % and 7.4/0 %, respectively. Elevated serum ALT levels were observed in 52 % of obese males and 33 % of obese females, while, in participants whose BMI was less than 22, 25 % of males and 4 % of females showed elevated serum ALT levels.**Discussion and conclusions:** Considering the etiology of liver disease in Japan, most of the participants studied with increased serum ALT levels were affected with NAFLD. Male adolescence seems to be more susceptible to NAFLD than females even when BMI indicates they are far from obesity.

P-0952

Serum immunoglobulin A level is a predictor for fibrosis in non-alcoholic fatty liver disease**Salih Boga¹, Huseyin Alkim¹, Ali R. Koksall¹, Mehmet Bayram¹, Ilker Sen¹, Muveddet B. Yilmaz Ozguven², Canan Alkim¹**¹Department of Gastroenterology, Sisli Hamidiye Etfal Education and Research Hospital, Istanbul, Turkey; ²Department of Pathology, Sisli Hamidiye Etfal Education and Research Hospital, Istanbul, Turkey**Aim:** Elevated immunoglobulin (Ig) A levels and its relationship with fibrosis in Alcoholic Liver Disease (ALD) were reported. Nonalcoholic fatty liver disease (NAFLD) shows similar histology and pathophysiology with ALD. We aimed to evaluate serum Ig (IgA, IgG, IgM) levels in biopsy-proven NAFLD patients and determine if Ig levels are associated with clinical or histological features.**Methods:** Seventy patients and 54 volunteers as controls seen in Sisli Hamidiye Etfal Education and Research Hospital between 2013 and 2014 were included.**Results:** There was no statistical difference between NAFLD vs. controls and nonalcoholic steatohepatitis (NASH) ($n = 53$) vs. non-NASH ($n = 17$) in terms of Ig levels. When NAFLD patients with normal and elevated IgA levels were compared (Table 1) NASH and DM ratios were higher in patients with elevated IgA levels. There was a significant positive correlation between serum IgA levels and the stage of fibrosis ($r = 0.636$, $p < 0.001$) (Figure 1). When NAFLD patients were compared as patients with no/mild fibrosis and patients with advanced fibrosis (Table 2), IgA, age, sex, HOMA-IR, and BMI were all significantly increased in advanced fibrosis. In logistic regression analysis, IgA and HOMA-IR were found to be independent predictors of advanced fibrosis. When IgA levels were evaluated by ROC analysis to differentiate advanced fibrosis from mild fibrosis AUC was 0.874. At the cut-off level of 391.5 mg/dl for IgA, sensitivity was 78.9 % and specificity was 88.2 %.**Conclusion:** The serum IgA levels showed a gradual increase with increasing fibrosis stages in NAFLD patients. The serum IgA was an independent predictor of hepatic fibrosis

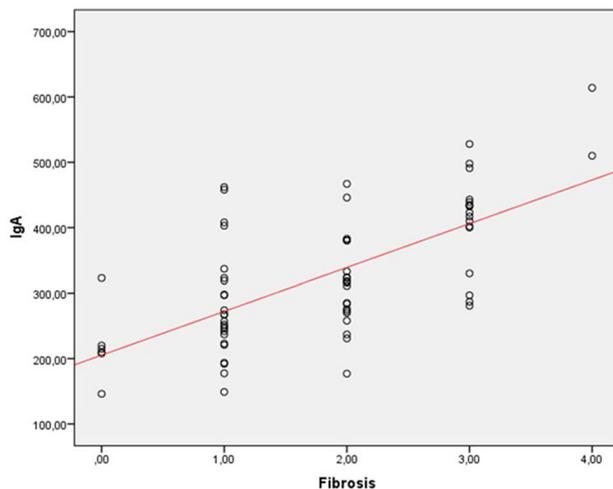


Figure 1: The correlation graphic showing the distribution of immunoglobulin A levels according to fibrosis stages.

TABLE 1:

	Elevated IgA (n=21)	Normal IgA (n=49)	p value
Age	47.7±9.5	43.7±11.9	0.166
sex, F/M	14/7	31/18	0.163
BMI, kg/m ²	31.4±5.0	29.5±5.4	0.161
ALT, IU/L	74 [49.5-95.5]	66.0 [39.5-104.0]	0.413
AST, IU/L	48.0 [41.0-64.5]	48.0 [33.5-71.0]	0.648
GGT, IU/L	45.0 [25.5-67.0]	19.5 [35.0-57.0]	0.197
Albumin, g/L	4.4±0.4	4.6±0.4	0.025
HOMA-IR	5.6 [3.3-7.0]	3.0 [1.9-4.6]	0.001
hs-CRP, mg/dL	4.5 [2.5-5.9]	3.3 [1.2-6.4]	0.276
Total cholesterol, mg/dL	201.0 [161-216.5]	192.0 [170.0-227.5]	0.844
LDL-C, mg/dL	128.0 [98.8-147.5]	114.6 [96.8-146.5]	0.718
HDL-C, mg/dL	41.0 [36.5-45]	47 [37.5-55.0]	0.025
TG, mg/dL	127.0 [90.5-170.0]	134.0 [96.0-204.0]	0.714
IgA, mg/dL	453.3±52.0	272.3±63.1	<0.001
IgG, mg/dL	1246.5±206.4	1217±267.6	0.658
IgM, mg/dL	100.1±52.2	99.8±40.8	0.981
NASH ratio, %	%52.9	%47.1	0.018
DM ratio, %	%71.4	%42.9	0.028

Values are presented using means ± standard deviations for normally distributed and medians and first and third quartiles in the brackets for the non-normally distributed variables. BMI: body mass index; ALT: alanine aminotransferase; AST: aspartate aminotransferase; GGT: gamma-glutamyl transpeptidase; HOMA-IR: homeostasis model assessment of insulin resistance, hs-CRP: high-sensitivity c-reactive protein LDL-C: low density lipoprotein cholesterol; HDL-C: high density lipoprotein cholesterol; TG: triglyceride, Ig: immunoglobulin, NASH: Non-alcoholic steatohepatitis, DM: Diabetes Mellitus

TABLE 2:

	No/Mild Fibrosis (Stage 0-2, n=51)	Advanced Fibrosis (Stage 3-4, n=19)	p value
Age	44.2±12.2	49.9±7.7	0.026
Sex, F/M	37/14	8/11	0.018
BMI, kg/m ²	30.7±4.1	33.2±4.6	0.031
ALT, IU/L	76.0 [53.0-116.0]	82.0 [49.0-102.0]	0.958
AST, IU/L	55.0 [40.0-71.0]	54.0 [40.0-89.0]	0.979
GGT, IU/L	48.0 [29.0-67.0]	37.0 [26.0-67.0]	0.579
Albumin, g/L	4.6±0.3	4.5±0.5	0.200
HOMA-IR	3.4 [2.6-4.8]	6.3 [5.8-6.8]	0.001
CRP, mg/dL	4.5 [2.2-6.9]	3.6 [2.1-5.9]	0.456
Total cholesterol, mg/dL	201.0 [172.0-234.0]	189.0 [157.0-217.0]	0.127
LDL-C, mg/dL	116.8 [98.8-150.0]	104.8 [94.0-147.0]	0.273
HDL-C, mg/dL	44.0 [36.0-53.0]	42.0 [38.0-47.0]	0.341
TG, mg/dL	151.0 [100.0-205.0]	127.0 [85.0-168.0]	0.210
IgA, mg/dL	287.6±79.5	424.8±85.2	<0.001
IgG, mg/dL	1245.5±280.0	1211.9±213.8	0.638
IgM, mg/dL	94.1±37.7	97.6±51.6	0.754

Values are presented using means ± standard deviations for normally distributed and medians and first and third quartiles in the brackets for the non-normally distributed variables. BMI: body mass index; ALT: alanine aminotransferase; AST: aspartate aminotransferase; GGT: gamma-glutamyl transpeptidase; HOMA-IR: homeostasis model assessment of insulin resistance, hs-CRP: high-sensitivity c-reactive protein LDL-C: low density lipoprotein cholesterol; HDL-C: high density lipoprotein cholesterol; TG: triglyceride, Ig: immunoglobulin, NASH: Non-alcoholic steatohepatitis, DM: Diabetes Mellitus

P-0953

To evaluate non-invasive predictors in diagnosis of nonalcoholic steatohepatitis

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Aim: To see the whether non-invasive markers like certain anthropometric and laboratory markers could predict steatohepatitis.

Method: Demographic, clinical, laboratory and histologic data were analyzed in 104 adult patients who were diagnosed with fatty liver by ultrasonogram examination and finally with histologic findings. Histological inflammation and fibrosis in NAFLD patients were examined with respect to age, gender, BMI, alanine aminotransferase (ALT) level, aspartate aminotransferase (AST) level, gamma -glutamyltransferase level, platelet count, serum ferritin level, presence of hypertension, diabetes, hypertriglyceridemia and insulin resistance.

Result: We enrolled 104 patients. The Kleiner scoring system was used to classify NAFLD. Among them 50 were diagnosed as

NNFL(48.1 %) and 54 were diagnosed as NASH(51.9 %). There is no significant difference in ALT level, AST level, serum ferritin level and, presence of high BMI, hypertension, hypertriglyceridemia, diabetes and insulin resistance in between NNFL and NASH

Conclusion: Histologic inflammation and fibrosis in NAFLD patients could not predict by non-invasive marker like level of ALT, AST, serum ferritin or with presence of metabolic syndrome.

P-0954

Effects of liver steatosis on the progression and the antiviral response in chronic hepatitis B

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Background and objective: Hepatitis B virus (HBV) infection is a major cause of liver cirrhosis and liver cancer in China. The study was to investigate the effect of liver steatosis on the prognosis and antiviral response of chronic hepatitis B (CHB) patients in prospective-retrospective nested case-control study.

Materials and methods: A total of 372 chronic hepatitis B cases (292 males and 80 females) confirmed by liver biopsy were selected. The patients were divided into CHB without steatosis ($n = 226$) and CHB with steatosis ($n = 146$). All subjects were followed up to 5 years. The clinical end point was diagnosis as cirrhosis or liver cancer.

Results: (1) In chronic hepatitis B with steatosis patients, the mean age, male proportion, BMI, cholesterol, and fibrosis stage were significantly higher than those in the group without steatosis at baseline ($P < 0.05$). (2) Fibrosis index APRI and S index were decreased in patients without steatosis ($P < 0.05$). The FIB-4 index and Forns index were decreased in patients without steatosis and were increased in chronic hepatitis B with steatosis patients ($P < 0.05$). (3) The 5-year cumulative incidence of cirrhosis was 5.19 % in patients without steatosis, and 4.17 % in chronic hepatitis B with steatosis patients, respectively, ($P > 0.05$). (4) The age and higher fibrosis stage were an independent risk factor (RR 1.065, $P = 0.045$).

Conclusion and statement: The baseline characteristics of chronic hepatitis B with steatosis were different from those without steatosis. The liver steatosis may effect on the progression in CHB patients. The age and higher fibrosis stage were independent risk factors in CHB patients development to cirrhosis.

P-0955

NASH challenges HBV as the leading cause of chronic hepatitis in Bangladesh

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Aim: Our study in 2007 (Mahtab et al, Indian J Gastroenterol) showed that HBV is the leading cause of chronic hepatitis in Bangladesh. Aim of present study was to see if there is any change in the etiology of chronic hepatitis in this country eight years down the line.

Methods: This is a retrospective study. Patients attending Hepatology Green Unit, Bangabandhu Sheikh Mujib Medical University, Dhaka in 2014 with chronic hepatitis were included.

Results: Total 3226 patients were included. Of them 62.4 [2012] cases were due to HBV, 34 % [1095] due to NASH, 2.5 % [81] due to HCV and 0.9 % [28] due to Wilson's disease 0.3 % [10] due to autoimmune hepatitis. However in case of females 53 % [494/932] due to NASH, while 40.6 % [378/932] due to HBV. Although overall HBV remains the leading cause of chronic hepatitis in Bangladesh (76.3 % in 2007), NASH (22.8 % in 2007) is increasingly becoming important as the leading etiology of chronic hepatitis in this country, especially in case of females.

Conclusion: HBV is the commonest cause of cirrhosis in Bangladesh. The government has integrated HBV vaccine into the existing Expanded Programme of Immunization. However we have to go a long way before we can sustain HBV in Bangladesh. Moreover with our economic growth, lifestyle-related disease like fatty liver is becoming an important concern.

P-0956

Nonalcoholic fatty liver disease is associated with erectile dysfunction: a prospective pilot study

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Introduction: Nonalcoholic fatty liver disease (NAFLD) is considered the hepatic manifestation of metabolic syndrome (MetS). Although the link between MetS and erectile dysfunction (ED) is well known, clinical studies investigating the association between NAFLD and ED are scant.

Methods: Male patients with biopsy-proven NAFLD were prospectively asked to fill the five-item International Index of Erectile Function (IIEF-5) questionnaire. Their clinical and histologic variables were compared with the IIEF scores.

Results: Forty male patients aged 33 (24–57) with biopsy-proven NAFLD had a median IIEF-5 score of 16 (9–25) and MetS was present in 23 (57.5 %). ED severity distributions as moderate, mild and no ED were 11 (27.5 %), 16 (40 %), and 13(32.5 %), respectively. Histological NAFLD score was significantly higher in patients having ED compared to patients with no ED (5.63 + 1.39 vs. 4.15 + 1.46; $p = 0.006$). MetS diagnosis was significantly more common in patients having ED, compared to those without ED [19 (70.4 %) vs. 4 (30.8 %), respectively, $p = 0.018$]. When patients with and without ED were compared gamma glutamyl transferase was significantly lower in ED while components of MetS did not correlate with ED. After multivariate analysis, NAFLD score has remained the only significant outcome associated with ED [$p = 0.01$; OR (95 % CI): 2.96 (1.266–6.899)].

Conclusions: The current clinical study demonstrates a significant association between NASH and ED for the first time. Our findings suggest liver damage may play role in the pathogenesis of ED in patients with NAFLD. Future studies are needed to expand the underlying common mechanisms responsible for this novel hypothesis.

P-0957

Non-invasive assessment of hepatic steatosis in patients with anorexia nervosa using elastography

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Background: Mild liver injury is a complication of anorexia nervosa (AN) in around 30 % of cases (Tomita K, Hepatol Res. 2014 44;E26-31). Part of patients with AN reported to present fatty liver change. Recently, noninvasive quantification of the hepatic steatosis using FibroScan has been reported in a variety of liver diseases. In this study, we conducted the FibroScan measurement in patients with AN.

Methods: Nine patients hospitalized at our institution with a diagnosis of AN were enrolled as the study subjects. All these patient received FibroScan test followed by normal ultrasonography. Liver fat contents were assessed using controlled attenuation parameter (CAP) software.

Results: All of the enrolled subjects were female with median age of 40 (19–72) years, and mean BMI 15.6 (12.0–18.1) kg/m². Their laboratory data was: average ALT; 41.9 ± 13.6 IU/L, γ -GTP; 51.5 ± 15.5 IU/L, TG; 97.4 ± 24.8 mg/dL and T-cho; 200.6 ± 14.4 mg/dL. Elevated liver enzyme (ALT level > 42 IU/L and γ -GTP > 47 IU/L) was observed in 2 (22 %) and 3 (33 %) of the 9 cases. The BUN/creatinine ratio were high in all of patients (mean BUN/creatinine: 28.8 ± 3.0). FibroScan measurements showed the median CAP as 190 (144–248) dB/m. Two patients who had high range (CAP > 237) had evident fatty liver with ultrasonography, although these patients did not have elevated liver enzyme.

Conclusions: Hepatic steatosis in patients with AN could be successfully assessed using FibroScan similar to traditional ultrasonography. FibroScan could detect hepatic steatosis even before the emergence of elevations of serum transaminases. Scoring the fatty liver using FibroScan may be beneficial to keep track of clinical course patients with AN

P-0958

Nonalcoholic fatty liver disease needs more metabolic abnormalities than polycystic ovary syndrome

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Background and aim: NAFLD and PCOS are two common metabolic diseases. We aimed to evaluate the underlying mechanisms in the development of both NAFLD and PCOS and then, compare with each other.

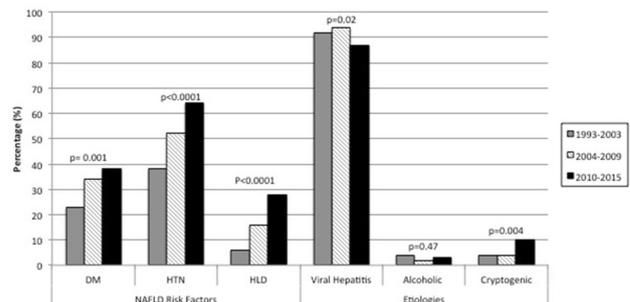
Patients and methods: Thirty female patients with NAFLD without PCOS; 12 female patients with PCOS and normal liver enzymes; and a control group with 17 healthy female were included.

Results: BMI in NAFLD (29.4 kg/m) were more than those in PCOS (25.6 kg/m). There was no difference between patients with PCOS and controls for BMI. The mean fasting insulin level in NAFLD was more than those in PCOS (5.06 unit more) and controls (12.8 unit

more). In NAFLD group; the mean serum levels of fasting glucose, insulin, triglyceride and cholesterol were higher than those in the PCOS group. Then, we evaluated insulin resistance and pancreas beta-cell function in 10 non-diabetic PCOS patients and 19 non-diabetic NAFLD patients. Insulin resistance measured by HOMA-IR was 4.1 in nondiabetic NAFLD and 2.7 in nondiabetic PCOS. Pancreas beta-cell function by HOMA-B was 509 % in NAFLD and 98 % in PCOS.

Discussion: Although liver as a insulin sensitive organ such as muscle and pancreas plays a central role in metabolic syndrome, our study showed that NAFLD needs more severe metabolic abnormalities than PCOS.

Figure 1: Prevalence of NAFLD risk factors and cirrhosis etiology from 1993 to 2015



P-0959

Study of hepatic steatosis Index in patients with chronic HCV infection

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Introduction: Hepatic steatosis is a frequent cause for elevated serum aminotransferase levels. The main screening method is ultrasonography. A combination of non-invasive tests may help in the diagnostic evaluation of a patient with suspected steatosis. Liver biopsy is the gold standard to precisely diagnose NASH. In this study we assessed the usefulness of hepatic steatosis index in comparison with ultrasonography and histopathological examination in the evaluation of steatosis associated with HCV infection.

Methods: We studied fifty HCV patients who have bright liver in ultrasound. All patients were further studied with histopathological examination of liver biopsy as well as lab investigations. Hepatic steatosis index was also calculated.

Results: A significant positive correlation has been found between hepatic steatosis index and Metavir staging of fibrosis assessed by histopathological examination. When the mean value of HSI = 35.5, the stage was F0 and when the mean value of HIS = 43.7, the stage was F3. A significant positive correlation has also been found between hepatic steatosis index and histopathological grading of steatosis. When the mean value of HSI = 31.7, the grade was G0 and when the mean value of HSI = 39.1, the grade was G3.

Conclusion: Hepatic steatosis index may offer an economical non-invasive tool for predicting of hepatic steatosis with reasonable accuracy. In practice, the index could be used for the detection of

risky patients in order to offer more advanced investigation, nutrition and life style counselling, as well as for the detection of severe steatotic patients requiring medical supervision and therapy.

P-0960

The association between chronic hepatitis B/C infection and metabolic syndrome

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Background: Chronic Hepatitis C (CHC) and Hepatitis B (CHB) virus infections are widely seen as major causes of chronic liver disease worldwide. Although the association between HCV and metabolic disturbances has been cited, scientific data supporting the same relation with HBV is scarce. The aim of this study was to conduct a head-to-head comparison between CHC and CHB in terms of association with metabolic syndrome (MS).

Methods: Sixty-two patients (22 males) with CHC (Group 1), and 358 patients (219 males) with CHB (Group 2) were included in the study retrospectively. Diagnosis of MS was made according to the criteria of the International Diabetes Federation. Insulin resistance (IR) was defined using the homeostasis model assessment index (HOMA).

Results: Twenty-four patients in Group 1 (38.7 %) and 97 patients in Group 2 (27.1 %) were diagnosed as MS ($p = 0,086$). The rate of patients with DM or patients with IR were similar in both groups, 11 (17.7 %), 32 (8.9 %) ($p = 0.059$), 31 (50 %), and 176 (49.2 %) respectively ($p = 0.987$). In both groups, the number of patients diagnosed with fatty liver by ultrasound were 24 (38.7 %) and 137 (38.3 %) respectively ($p = 0.939$).

Conclusions: The results suggest that CHB infection association with MS is similar to the association found between CHC infection and MS. Although the study indicates a significant association between CHB infection and MS, additional studies are needed to confirm these findings.

P-0961

Comparison of complications between nonalcoholic fatty liver disease vs alcoholic liver disease

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Background: Nonalcoholic fatty liver disease (NAFLD) and alcoholic liver disease (ALD) show similar liver histology but different clinical features. Here, we compared the incidence of hepatic and extrahepatic complications between NAFLD and ALD.

Methods: 806 patients with NAFLD and 584 patients with ALD were diagnosed with liver cirrhosis (LC) based on liver biopsy. We retrospectively assessed the cause of 75 cirrhotic NAFLD patients and 103 cirrhotic ALD patients.

Results: (1) HCC incidence: After age and gender adjustments, the 5-year HCC development rate was 11 % in the NAFLD-LC group and 9 % in the ALD-LC group. (2) Incidence of extrahepatic

malignancies: The 5-year extrahepatic malignancy development rate in the ALD-LC group (9 %) was significantly higher than that of the NAFLD-group (4 %). (3) Complication rate and treatment of esophago-gastric varices: 43 of the 64 NAFLD-LC patients and 34 of the 61 ALD-LC patients had esophago-gastric varices. Additionally, 24 % of the NAFLD-LC patients and 44 % of the ALD-LC patients had high-risk varices defined by red spots and F2 (nodular) or F3 (large or coiled) lesions. (4) Overall survival rate and cause of death: The 5-year survival rate was 89 % in both two groups. 19 NAFLD-LC patients died due to liver-related disease (74 %), whereas 20 ALD-LC patients died due to liver-related disease (40 %).

Conclusion: The rate of survival and hepatic malignancy were similar between the two diseases, but the rate of esophago-gastric varices, extrahepatic malignancy, and death due to extrahepatic diseases differed. When treating ALD-LC patients, clinicians should monitor the possibility of extrahepatic diseases.

P-0962

Utility of quantitative controlled attenuation parameter to diagnose alcoholic liver disease

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Introduction: Although alcoholic liver disease (ALD) and non-alcoholic fatty liver disease (NAFLD) tend to be gathered as non-viral liver disease because the similar specific liver tissue, the natural history and etiology are considerably different between them. We need to distinguish both of them to do appropriate treatment intervention. Questioning of amount of drinking is needed, but we experience some difficult cases to understand drinking history because of a too little declaration of amount of drinking. In this study, we aimed to investigate the diagnostic performance of controlled attenuation parameter (CAP) based on Fibroscan@502 (Echosens) in ALD patients.

Method: Non-viral hepatitis patients who intake alcohol more than 60 g ethanol as ALD (63 cases) and less than 20 g per day as NAFLD (177 cases) were enrolled. Fatty liver Index (FLI) (Bedogni G.2006), Hepatic steatosis index (HSI) (Lee JH.2010) and Lipid accumulation product (LAP) (Chinag Jui-Kun.2012) known as liver steatosis prediction formula from various physical measurement and multiple blood markers were investigated. CAP was performed simultaneously. We analyzed whether CAP was useful for detecting ALD compared with NAFLD.

Results: ALT, HSI and CAP were significantly lower, γ GTP was higher in the ALD group compared with NAFLD group. A multivariate statistics with these 4 markers was investigated, γ GTP (OR = 1.009, $p < 0.0001$) and CAP (OR = 0.989, $p = 0.0007$) were important parameters to distinguish between them.

Conclusion: Comparing the results of ALD patients to those of NAFLD patients, we elucidate the diagnostic accuracy of CAP for ALD patients.

P-0963

Systematic review: Steroid, pentoxifylline or combined therapy for acute alcoholic hepatitis

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Background: Even though both of corticosteroid and pentoxifylline are currently recommended drugs for the treatment of severe alcoholic hepatitis, it is still unclear that they are really effective in reducing mortality. This systematic review aims to evaluate the therapeutic effect of these drugs to guide proper treatment in the patients with severe alcoholic hepatitis using Cochrane methods.

Methods: We used multiple comprehensive databases to find randomized controlled trials comparing therapeutic effect of corticosteroid, pentoxifylline and dual therapy of corticosteroid plus pentoxifylline in alcoholic hepatitis.

Results: Total 2639 patients from twenty five studies were analyzed. In overall mortality, there was no difference at mortality among each therapeutic modality. When 28-day mortality was analyzed, corticosteroid monotherapy reduced mortality compared to placebo control (OR 0.58; 95 % CI 0.34–0.98, $p = 0.04$), but pentoxifylline monotherapy did not (OR 0.60; 95 % CI 0.31–1.19, $p = 0.15$). Interestingly, dual therapy showed similar mortality compared to corticosteroid monotherapy (OR 0.92; 95 % CI 0.63–1.35, $p = 0.68$), but significantly decreased incidence of hepatorenal syndrome (OR 0.47; CI 0.26–0.86, $p = 0.01$) and risk of infection (OR 0.63; CI 0.41–0.97, $p = 0.04$) than corticosteroid monotherapy. However, there were no medium- or long-term survival benefit in all kind of the treatment arms.

Conclusions: Corticosteroid could decrease short-term mortality in patients with severe alcoholic hepatitis and adding pentoxifylline to corticosteroid might be best option because dual therapy was not inferior compared to corticosteroid monotherapy, as well as it could reduce development of hepatorenal syndrome and decrease risk of infection.

P-0964

Clinical factors correlated to alcohol withdrawal in patients with alcoholic liver disease

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Background: Alcohol related diseases including alcohol withdrawal (AW) are still big social and medical problems. But the studies about clinical factors associated with the development and prognosis of alcohol withdrawal are lacking. Therefore, the aim of this study was to evaluate the clinical factors associated with AW in patients who were admitted to a general hospital with alcoholic liver disease (ALD).

Method: This retrospective case-control study targeted the patients with ALD who were admitted in the Kangwon national university hospital between January 2008 and October 2013. We divided two groups, AW and non-AW, and analyzed alcohol consumption habits, comorbidities, laboratory findings and hospital courses by medical record.

Results: We analyzed 212 cases out of 602 admitted cases, 390 cases were excluded due to alcohol abstinence for more than 1 month before admission. In AW group, 54 cases (25.4 %) were included and median age was 49.7 years old. Presence of previous alcohol withdrawal history ($p = 0.036$), higher diastolic pressure ($p = 0.003$), more elevated MCV ($p = 0.021$), and GGT ($p = 0.000$) and less PT(INR) ($p = 0.030$) were statistically significant variables between two groups. In addition, AW patients needed a longer ICU stay and length of hospitalization compared to the patients without AW.

Conclusion: Prior alcohol withdrawal history, MCV, GGT, and PT(INR) were associated with AW. And there were no significant differences in mortality rates between two groups, but longer ICU stay and length of hospitalization were required for management of AW. Therefore, a proper evaluation and treatment is needed as soon as they are admitted.

P-0965

The comparison examination with a serum sodium level and varicose recurrence in alcoholic cirrhosis

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Objectives: The hemorrhage from ruptured esophagogastric varicose vein is one of the main causes of death in alcoholic cirrhosis. Several studies have shown that serum sodium level (sNa) correlates with the prognosis for patients with liver cirrhosis. It is still unknown if hyponatremia is associated with the therapeutic effect of endoscopic varicose treatment. Here, we show the relationship between sNa and the esophagogastric varicose vein recurrence after endoscopic treatment in alcoholic liver cirrhosis.

Method: We selected 46 cases have the esophagogastric varicose vein treatment from 2009 through 2013 in alcoholic liver cirrhosis, and evaluated the correlation with the sNa and serum data, oral treatment such as a diuretic and the branched chain amino acid (BCAA) or the rate of the varicose vein recurrence among one year.

Results: The average age of patients was common in men at 61.9 years old. 58.3 % of patients have EVL and 40.9 % of EIS needed retreatment for esophagogastric varicose vein within one year. Whereas 23.5 % of patients with sNa > 140 mEq/L and 62.1 % of patients with sNa < 140 mEq/L needed retreatment for esophagogastric varicose vein within one year. Serum albumin and PT activity in patients with sNa < 140 mEq/L were significantly lower than sNa > 140 mEq/L. Patients with sNa < 140 mEq/L mostly receive the medication of diuretic or BCAA.

Conclusions: Patients with sNa < 140 mEq/L may indicate the high risk of varicose vein recurrence after endoscopic treatment. The sNa was valuable information for predicting the effect of endoscopic varicose treatment.

P-0966

BIA is in proximate agreement with CT based assessment of sarcopenia in ALD patients**Varsha shasthry, Jaya Benjamin, Chetan Kalal, Guresh Kumar, Shiv_Kumar Sarin, Yogendra_Kumar Joshi**

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Background: Sarcopenia is associated with morbidity and mortality in patients with alcoholic liver disease (ALD). Despite computed tomography (CT) being ideal for assessment of sarcopenia, is expensive and cumbersome thus necessitating identification of other easy and inexpensive yet objective methods.

Aim: To determine the agreement between various indirect methods and CT for the assessment of sarcopenia.

Methods: Muscle mass measurements in patients with ALD was done by lumbar site CT (Discovery 750HD; GE), DEXA(Discovery; Hologic), BIA(Multifrequency; MC-180;TANITA), mid arm muscle circumference (MAMC). Using previously derived CT based SMI (cm^2/m^2) cut-offs at 1SD and 2SD below the mean of 120 male healthy controls (MHC); class I and class II sarcopenia were identified and the corresponding cut-offs of other methods were derived by plotting the receiver operating characteristic (ROC)curve. The agreement between the Z scores of various methods was assessed by intra-class coefficient correlation (ICC).

Results: Muscle mass in ALD patients (n = 50; M: 100 %; age 42.8 ± 8.6 years; BMI 23.7 Kg/m^2 , Child A: B: C = 17 %:43 %:40 %) were studied. The cut-offs of SMI assessed by CT in MHC were 36.1 ($<-2\text{SD}$) and 43.7 ($<-1\text{SD}$) (unpublished data) and the prevalence of Class2 and Class1 sarcopenia in patients with ALD was 10 % and 28 % respectively. Of all methods, BCA-total SMI and phase angle showed substantial (AUC >0.77) agreement with the gold standard CT and perfect agreement (ICC >0.73 ; $p < 0.001$) in Z scores with CT.

Conclusion: Bioelectrical impedance analysis based evaluation of sarcopenia is a reliable and alternative method and in close agreement with the gold standard method CT in patients with ALD.

P-0967

Prednisolone plus S-adenosil-L-methionine in severe alcoholic hepatitis**Petr Tkachenko, Inna Komkova, Marina Maevskaya, Vladimir Ivashkin**

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Background: Severe alcoholic hepatitis (AH) is a life-threatening liver disease with a potential of 30–40 % mortality at 1 month. While steroids remain to be a first line therapy, they provide only about 50 % survival benefit. The aim of the study was to evaluate the efficacy of glucocorticoids plus S-adenosyl-L-methionine (SAME), as compared to glucocorticoids alone, in patients with severe alcoholic hepatitis.

Methods: 40 patients with severe AH were randomized in 2 groups and enrolled in the prospective trial. Group 1 (n = 20) patients received prednisolone 40 mg/daily per os, group 2 (n = 20) patients were managed with prednisolone 40 mg/daily per os plus SAME 800 mg i.v. Treatment duration was 28 days.

Results: The response rate assessed by Lille model was significantly higher in prednisolone plus SAME group (19 of 20; 95 %), than in prednisolone group (13 of 20; 65 %), $p = 0.044$. 2 patients died, all

from prednisolone group (10 %). There were no lethal outcomes in prednisolone plus SAME group. Kaplan-Meier method showed no significant differences between two groups ($p = 0.151$, Log-Rank). Hepatorenal syndrome (HRS) occurred in 20 % in the prednisolone group (4 of 20 patients) while no HRS cases were registered in prednisolone plus SAME group ($p = 0.035$).

Conclusions: Management of severe alcoholic hepatitis with prednisolone plus SAME was associated with better therapy response ($p = 0.044$) and less frequent HRS occurrence ($p = 0.035$). Mortality was not significantly lower in the prednisolone-SAME group than in the prednisolone-only group at 28 days (10 % vs. 0 %, $p = 0.151$).

P-0968

Remnant liver ischemia is associated with early recurrence and poorer survival after hepatectomy**Jaiyoung Cho, Ho-Seong Han, YoungRok Choi, Yoo-Seok Yoon, Jae Yool Jang, Hanlim Choi, Jae Seong Jang, Seong Uk Kwon**

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Background: Non-anatomical hepatectomy or compromised blood supply to the remnant liver may result in remnant liver ischemia (RLI) of various extension and severity. There are a few reports showing association between ischemia-reperfusion injury and tumor recurrence in animal liver transplantation model, however, reports to evaluate the impact of ischemia on patient survival after hepatectomy are scarce.

Method: RLI was graded on postoperative CT scan in 328 patients who underwent hepatectomy for hepatocellular carcinoma (HCC) between January 2004 and December 2013. We defined RLI as reduced or absent contrast enhancement during the venous phase and classified to minimal (none or marginal) or severe (partial, segmental, and necrosis).

Results: We observed radiologic signs of severe RLI in 98 patients (29.9 %): 63 partial, 16 segmental, and 19 necrosis, and these patients showed more complications ($P < 0.001$) and longer hospital stays ($P = 0.002$). Preoperative history of transarterial embolization ($P = 0.040$), use of Pringle maneuver ($P = 0.028$), and longer operation time ($P < 0.001$) were independent risk factors for developing RLI. Patients with severe RLI showed higher rates of early recurrence within 6 or 12 months after hepatectomy compared those without ($P < 0.001$). Moreover, RLI was independent risk factors for both overall patient ($P < 0.001$; OR = 6.984; 95 % CI, 4.268–11.426) and disease-free survival ($P < 0.001$; OR = 5.153; 95 % CI, 3.615–7.345).

Conclusion: Prevention and technical refinement in partial hepatectomy without RLI should be directed to improve survival in patients with HCC.

P-0969

Successful laparoscopic deroofing for liver cysts: our technique and outcome**Toshio Shikano, Toshihiro Mori**

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Aim: With advances in laparoscopic surgery, laparoscopic deroofing has gained wide acceptance in the surgical community to treat

symptomatic non-parasitic hepatic cysts. Published non-surgical data still favour aspiration and sclerotherapy as treatment in these cases, though morbidity is higher and recurrence rates are not acceptable. We reviewed all patients that had been treated by laparoscopic deroofing in our department over a period of 6 years in order to find out if the surgical approach should be considered the standard treatment.

Materials and methods: From November 2012 to August 2015, 12 laparoscopic deroofings were performed in 6 patients with symptomatic cysts. All patients were followed up, and morphologic evaluation was performed with repeated abdominal US and CT.

Results: All operations could be finished laparoscopically without converting to open laparotomy. Intra- and postoperative complications were not detected. Mean operation time was 89 min (range 37–160 min), blood loss was 5 ml (range 0–18 ml), post-operative hospital stay was 3.2 days (range 2–4 days), size of treated cysts was 16.7 cm (range 14–20 cm). Follow up showed no symptomatic recurrence after surgery.

Conclusion: Laparoscopic deroofing of hepatic cysts is a safe and effective treatment option. Our data suggest that the risk of operation is justified and that the method is superior to sclerotherapy.

P-0970

Effect of introducing an ERAS for patients undergoing resection of HCC

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Background: Enhanced recovery after surgery (ERAS) programs following surgical interventions are within the standard of care for patients with several surgical indications, but their value in liver surgery remains uncertain. This study analyzed whether an ERAS program for patients undergoing potentially curative liver resection for hepatocellular carcinoma (HCC) influenced the feasibility, safety, and effectiveness of surgery.

Methods: Clinicopathologic factors, surgical factors, and outcomes were compared in patients who underwent extended hepatectomy for HCC before and after the introduction of an ERAS program.

Results: Operating time and postoperative hospital stay were significantly shorter, and total volume infused during surgery significantly lower, for the ERAS than for the control group. Although the percentage of patients with retention of abdominal drainage was significantly smaller in the ERAS group, the frequency of abdominal paracentesis in patients without intraoperative abdominal drainage was higher in this group. Oral dietary intake and ability to walk stably occurred significantly earlier in the ERAS group. Postoperative serum concentrations of albumin and cholinesterase were significantly higher in the ERAS than in the control group.

Conclusions: The ERAS program for patients with mild to moderate liver dysfunction undergoing extended liver resection for HCC was feasible and effective. It allows earlier oral dietary intake, promotes faster postoperative recovery, and reduces hospital stay.

P-0971

Validation of a complexity classification to define surgical difficulty in patients with HCC

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Background: A classification system for defining the complexity of hepatectomy according to its technical difficulty was recently proposed as a consensus of a panel of experts. We validated this classification system for a prospective liver resection cohort in patients with hepatocellular carcinoma (HCC).

Method: The complexity classification separated liver resections into three categories of complexity (low, medium, or high complexity). We retrospectively reviewed 150 open hepatectomies between March 1, 2004 and November 30, 2013 in patients with HCC and compared the perioperative outcomes according to the complexity classification.

Results: There were no differences in patient demographics or pathologic findings among the three groups according to the complexity classification. The complexity classification effectively differentiated the three groups in terms of intraoperative findings and short-term outcomes. The mean estimated blood loss ($P = 0.001$), rate of blood transfusion ($P < 0.001$), and the mean operation time ($P < 0.001$) were significantly different among the three groups. The rates of overall and major complications ($P = 0.026$ and 0.005 , respectively) were significantly greater in the high complexity group. Multivariate analysis showed that the complexity classification was independently associated with major complications (OR = 4.73; $P = 0.040$). However, overall patient survival ($P = 0.139$) and disease-free survival ($P = 0.076$) were not significantly different among the three groups.

Conclusion: The complexity classification effectively differentiated intraoperative and short-term outcomes, and was independently associated with major complications after hepatectomy in patients with HCC.

P-0972

C3a receptor blockade reduces liver regeneration post-hepatectomy in LPS-hyporesponsive C3H/HeJ mice

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In the current investigation, we compared the effect of complement C3a-receptor blockade on the liver regeneration after 70 % partial hepatectomy (PH) in wild type C3H/HeN and the LPS-hyporesponsive C3H/HeJ mice.

Methods: 8–12 week old C3H/HeN and C3H/HeJ mice either received a specific C3aR-antagonist SB290157 (1mg/kg i.p.), or vehicle 45 min before surgery, and daily thereafter. Animals were studied at days 2, 3 and 7 post-PH. Liver weight/body weight ratio was calculated, and plasma C3a, C5a and IL-6 levels were measured by ELISA.

Results: The C3H/HeJ mice showed delayed restoration of liver mass compared to the C3H/HeN mice. C3aR-antagonist treatment attenuated liver regeneration in both C3H/HeN mice and C3H/HeJ mice, with a higher degree of attenuation seen in C3H/HeJ mice. C3a and C5a levels were elevated in both C3H/HeN and C3H/HeJ mice at day 2 (66–100 %; $p < 0.001$) and 3 (24–113 %; $p < 0.001$) post PH. C3aR blockade had no significant effect on C3a and C5a levels at any time-point. IL-6 was elevated at day 2 (1350–2300 %; $p < 0.001$) post-PH in both HeN and HeJ mice. Blockade of the C3aR caused a significant reduction of IL-6 at day 2 ($p < 0.001$).

Conclusion: Liver regeneration is delayed in the C3H/HeJ mice compared with control C3H/HeN mice. SB290157 treatment further reduced the regeneration. In the C3H/HeJ mouse, little restoration of liver mass had occurred by 7 days. Our results show that the complement system has a role in the liver regeneration, but would not appear to account for the delayed regeneration in the LPS-hyporesponsive C3H/HeJ mouse.

P-0973

HBIG free regimen for preventing post transplant hepatitis B recurrence

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Background: We report our center's experience with using HBIG free regimens as post transplant prophylaxis in patients with HBV infection.

Methods: Out of a total of 250 liver transplants 26 were done for HBV related liver diseases from March 2010 till date. The protocol at the institute is nucleos(t) ide based antiviral therapy. No HBIG was given. HbsAg, anti HBs and HBV DNA were tested at baseline and 6 monthly follow up.

Results: The median age was 45 years (32–51) (84.6 % males). Eleven transplants were done for cirrhosis, 6 for acute liver failure, 5 for acute on chronic liver failure and 4 for hepatocellular carcinoma. HBV DNA positive pre transplant was positive in 65 %. The baseline median HBV viral load was 269.5 IU/ml (0–3477.5). The patients were grouped in to 4 groups according to the antiviral given: Group 1- Tenofovir, group 2- Entecavir, group 3- Telbivudine and group 4- dual therapy (Tenofovir + Telbivudine or Entecavir + Telbivudine). The median time for viral clearance in group 1 (n = 13) was 6 months (1–24), group 2 (n = 4) was 6 months (1–12), group 3 (n = 2) was 6 months (1–12) and in group 4 (n = 5) was 6 months (1–12). There was no significant advantage of dual over single drug therapy for time to viral clearance (p = 0.44). The 5 years overall survival was 84.6 %.

Conclusion: With the availability of newer antiviral drugs with high genetic barrier to resistance, it is possible to recommend HBIG free regimens as post LT HBV prophylaxis.

P-0974

Modelling of hepatic flow variations due to liver resection and transplantation

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Liver transplantation is the treatment of choice for patients with end-stage liver disease. However, there is a huge and growing disparity between supply and demand for cadaveric liver donors. One solution which could solve this problem is the living donor liver transplantation (LDLT), whereby a portion of living donor's liver is resected and transplanted to a recipient. This procedure however is associated with

drastic flow alterations and increased risks of donor and recipient mortality. A good understanding of post-operative circulation in the liver is essential for successful LDLT procedures. In the Auckland Virtual Liver project bioengineers and clinicians are working together to develop multi-dimensional circulation models for the liver, where the hepatic flow models range from the organ level to sinusoidal level, i.e. from meter to micro-meter scales. Systemic control of hepatic circulations including the hepatic arterial buffer response (HABR) is coupled with the micro-circulation models in lobules, which allows us to investigate multiple flow phenomena due to a major liver resection or a lobectomy. Moreover, a novel bounding-box based segmental anatomy model is used to locate blood vessels to be removed so that surgical scenarios can be simulated. In particular, we applied this method to patient-specific cases where liver regeneration could be monitored through MRI imaging, and where adult-to-adult LDLTs could lead to small-for-size-syndrome in both donors and recipients. Wherever possible the computational model was compared with peri-surgical ultrasonic measurements for validation. The presented model thus yields new insights in hepatic circulation for liver transplantation.

P-0975

Reducing the rates of invasive CMV disease in post liver transplant patients

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Introduction: Cytomegalovirus (CMV) is the commonest and single most important viral infection in solid organ transplant recipients. Invasive CMV disease is associated with increased morbidity, mortality, and costs from end organ damage or complications of treatment. Sun et al reported a 1 % incidence of invasive CMV disease. Our centre, which adopts a pre-emptive strategy, showed a 10 % rate of invasive CMV disease. Our aim was to analyze key factors that led to high rates of invasive CMV disease, and to address them to reduce its incidence.

Methods: A project was undertaken to analyze the factors leading to increased incidence of invasive CMV disease. The team analyzing the factors was multi-disciplinary, consisting of members involved in post-transplant care. Key factors identified were (i) knowledge deficits among some members of the healthcare team, (ii) lack of access to a standardized protocol, (iii) lack of standardized communication, (iv) lack of medication reviews at each transition of care and (v) poor understanding of CMV by patients. Interventions were taken to address the above, with (i) continual education programmes for junior members of the team, (ii) placing the department protocol within easy access, (iii) preparation of a summary communication sheet, (iv) dedicated pharmacist to perform medication reconciliation, and (v) intensifying CMV disease counseling to pre-transplant patients.

Results: After implementation of the above protocols, there was a 10 % reduction seen in the annual incidence of invasive CMV disease. The annual incidence of invasive CMV disease dropped from 10 % (2013–2014) to 0 (2014–2015).

Conclusion: Significant reduction in invasive CMV in post liver transplant patients is achievable with a structured programme.

P-0976

The start of liver transplantation at the public hospital in Korea

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Background: We started a public hospital LT program to try to reduce patients' burden of expenses.

Methods: Twelve LTs were performed in Seoul Metropolitan Government Seoul National University Boramae Medical Center between July 2011 and March 2015.

Results: Six patients underwent LT from living donor and 6 patients underwent emergent LT from deceased donor. HBV-related liver disease (n = 6, 50.0 %) was the most common original disease followed by alcoholic liver disease (n = 4, 33.3 %). The median hospital stay was 21 (9–103) days. There was no hospital mortality. The total medical expense of LTs was 57,356,388 won (about 52,140 dollars) from operation to discharge and the expense paid by patients was only 13,378,735 won (about 12,160 dollars) under the health insurance coverage. There is one patient death during follow-up. The patient was 45-year-old female and underwent deceased donor LT for alcoholic liver cirrhosis. She was discharged on the postoperative 30th day without any major complication. However, she did not follow the medical instructions after discharge. Finally, she expired due to spontaneous bacterial peritonitis, progressed to septic shock at 10 months post-LT.

Conclusions: Our program showed that LT at the public hospital could decrease the expense of LT to the acceptable level with comparable results. However, the portion of alcoholic liver disease is higher in the public hospital, which could show poor outcome even after LT. Therefore, the established LT guideline for alcoholic patients including indication criteria and post-LT supporting program are needed for more expansion of LT at the public hospital.

P-0977

Polypharmacy and medication adherence in liver transplant candidates

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Background: Little is known about medication burden and adherence in the liver transplant (LT) setting.

Methods: To evaluate med adherence in LT candidates, we assessed medication adherence at a single center using the 8-item Morisky Medication Adherence Scale (MMAS; range 0–8; <6 = low adherence). Medication regimen complexity was assessed using the Medication Complexity Regimen tool (MRCI; based on form, frequency, and additional directions; no upper limit but average MRCI score of diabetic = 23). Chi-square or Wilcoxon rank sum tests were used to test differences in characteristics. Logistic regression was performed to determine associations with med adherence.

Results: Of 77 cirrhotics: 35 % female, median age 62y, MELD 13. Median [interquartile range (IQR)] #medications was 10 (7–14); MRCI was 21 (15–28). 45 % reported sometimes forgetting to take meds, 88 % reported administering meds themselves. By the MMAS, 25 (32 %) met criteria for low adherence (<6). 22 % reported sometimes not taking their medications within the past 2 weeks. The most common reasons for not taking medications were: 1) forgetfulness (28 %), and 2) timing issues (19 %). Lactulose was the most common medication that patients reported as least likely to take (24 %). Med adherence was associated with MELD (OR 1.09, 95 % CI 1.00–1.18) but not with # of medications, MRCI, age, race, or gender.

Conclusion: Nearly one-third of LT candidates display low adherence to their medications, which was independent of # of medications and medication regimen complexity. Our data underscore the need to develop strategies to increase medication adherence pre-transplant—which will remain in place post-transplant.

P-0978

The association between tumor necrosis factor-alpha polymorphism and acute solid organ rejection

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Currently, the association between tumor necrosis factor-alpha gene polymorphisms and the risk of acute rejection (AR) of a solid organ allograft is unclear. Therefore, we performed a meta-analysis to investigate this association. Forty-five case-control studies were included in this meta-analysis. The pooled p-value, OR and 95 % CI were used to measure the strength of the association. The A allele and genotypes including the A allele were significantly associated with AR susceptibility in solid organ recipients (A allele vs. G allele, random model, OR = 1.379, 95 % CI = 1.082–1.759, p = 0.009; A/A + A/G genotypes vs. G/G genotype, random model, OR = 1.413, 95 % CI = 1.077–1.854, p = 0.013; A/A genotype vs. A/G + G/G genotypes, fixed model, OR = 1.516, 95 % CI = 1.032–2.227, p = 0.034, respectively). The A/A genotype was associated with AR risk in liver transplantation recipients (fixed model, OR = 2.975, 95 % CI = 1.322–6.693, p = 0.008). This meta-analysis found that one particular tumor necrosis factor-alpha polymorphism (–308, G/A) may increase the risk for acute rejection. In the future, larger studies are needed to substantiate these findings.

P-0979

Surgical results of outflow reconstruction in living donor liver transplantation

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This study was done to assess the complication and patency rates of outflow reconstruction after living donor liver transplantation (LDLT) using modified right lobe (mRL) graft at a small volume center.

Materials: Consecutive 30 patients who received mRL graft during the period from April 2010 to December 2014 at Ulsan University Hospital were eligible for this study.

Surgical techniques: In 28 cases, recipient right hepatic vein (RHV) was extended to the IVC to match the length of donor RHV which was widened by fencing with homograft. Inferior right hepatic vein (iRHV) anastomosis was done in 80 % (24/30). When 2 or more iRHVs were present (4 cases), the openings were unified with homograft fence. Middle hepatic vein branches of segment 5 (V5) and 8 (V8) were reconstructed in all the cases (V5 in 29 cases and V8 in 26) with homograft (26), autologous great saphenous vein (2) or Gortex (2). The authors tried to make the anastomosis as short as possible in every case.

Results: There was no hospital mortality. No intervention for problems related with outflow was done so far. RHV anastomosis was anatomically and functionally stable in 100 % of the patients. Patency rate for iRHV anastomosis was 90 % (19/21) at 6 months. Patency rate for V5/V8 anastomosis was 87 % (26/30) at 1 month, during which the graft can regenerate enough to fulfill the metabolic needs of the recipients.

Conclusion: International knowledge sharing and education can make surgical results of LDLT satisfactory even for a small volume center.

P-0980

Liver transplantation in adults in Viet Duc University Hospital: a single-center experience

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Viet Duc University Hospital had the highest number of liver transplants in adults in Viet Nam. This presentation reports this single-center experience, highlighting the indications and initial results of the recipients.

Methods: Our study analyzed indications, techniques, early complications and results of 23 adult recipients who were performed liver transplantation from 2007 to 2015.

Results: There were 22 males, 1 female with 78.3 % were positive to HBV, 13 % was positive to HCV and 8.7 % were alcoholic liver diseases. HCC were the most common indications for liver transplantation with 18 patients and indication of decompensated cirrhosis in 5 patients. 3 of 23 cases were living donor transplantation and 20 were deceased donor transplantation without portacaval shunts. Immunosuppression pivoted on tacrolimus. Early complications were mainly with pleural effusions and acute rejections. 3 cases required relaparotomy and mortality in 1 case. Overall, there were 10 patients (43.4 %) had Clavien grade 0-2 complications, 12 patients (52.3 %) had Clavien grade 3 complications and 1 patient (4.3 %) had Clavien grade 5 complications

Conclusions: Liver transplantation in adults remains developing in Viet Nam. Viet Duc University hospital had initial experience in liver transplant surgery and postoperative care allowed standardization of the procedure in Viet Nam.

P-0981

Acute kidney injury after liver transplantation does not affect the patients' mortality

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Introduction: Acute kidney injury (AKI) after liver transplantation is not uncommon. Several literatures have shown a greater number of complications and higher mortality rates for patients with AKI after liver transplantation. The goal of this study was to determine the incidence of AKI during the early posttransplant period and evaluate the effects of AKI on mortality.

Patients and methods: We retrospectively reviewed the medical records of the patients aged “>” 18 years who underwent liver transplantation from March 2002 to September 2013. AKI was defined as an elevation of serum creatinine 1.5 times above the baseline or an absolute serum creatinine level >2 mg/dL. The exclusion criteria were hepatorenal syndrome at the time of transplantation and chronic renal failure with hemodialysis before liver transplantation.

Results: Of the 70 selected patients, 20 patients (28.6 %) developed AKI after liver transplantation, with 7 patients (35 %) requiring renal replacement therapy (RRT). All the patients with AKI requiring renal replacement therapy could be weaned from hemodialysis. 1-year survival rates were 90 and 80 % for patients without AKI and with AKI, respectively. But, the difference was not significant statistically ($p = 0.265$; odds ratio, 2.25). For patients who underwent RRT, the 1-month mortality rate and the 1-year mortality rate were significantly higher compared to the other patients who did not need RRT.

Conclusions: There was a high incidence of AKI in patients undergoing liver transplantation. There was no survival difference between AKI group and no-AKI group. But patients who required RRT showed significantly lower survival rates.

P-0982

Urinary-NGAL as a predictor of acute kidney injury in post-liver transplant recipients

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The aim is to study the value of u-NGAL as an early predictor for occurrence of AKI in patients undergoing liver transplantation (LT) in comparison with SCr and RIFLE classification.

Background: Renal dysfunction is common after LT. The incidence of AKI complicating the post transplant period is high and affects both short-term and long-term outcome. The difficulties in early intervention contribute significantly to the poor prognosis of AKI. This study was carried on 30 hepatic patients underwent living donor LT in the National Liver Institute, Menoufia University, Egypt, after history taking, clinical examination and preoperative routine investigations, and baseline renal functions were assessed. Operative data were collected during operation, patient were also assessed 5 days postoperatively for the occurrence of AKI by SCr, urine output and RIFLE classification vs u-NGAL.

Results: 14/30 patients (46.7 %) had AKI according to RIFLE classification, in those patients there was a significant relation between RIFLE classification and the cause of LT, preoperative platelet count, the use of basiliximab in the induction of immunosuppression and both day 1, day 2 u-NGAL. While u-NGAL levels above the cut-off value of 1300 pg/ml at day 1 and 4440 pg/ml at day 2 are considered good predictors of AKI post-LT with AUROC 0.77 and 0.77 respectively, and correlate significantly with different preop. and operative parameters.

Conclusion: u-NGAL is a valuable marker for early detection of AKI in patients undergoing LT before rise of SCr. levels above the cut-off value (1300 pg/ml) have a high sensitivity, specificity and positive predictive value for AKI post-LT

P-0983

Exacerbation of fatty liver ischemic reperfusion injury through NADPH oxidase signal transduction

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Introduction: Ischemic reperfusion injury of fatty liver is very severe, and fatty liver is inappropriate for donor graft in liver transplantation. Fatty liver is the most main reason of rejecting graft for deceased donor liver transplantation. Although NADPH oxidase (NOX) and toll like receptor (TLR) have been reported as the mechanism of ischemic reperfusion liver injury, there have been no studies that have evaluated the effects of ischemic reperfusion injury of fatty liver.

Methods: A rat model of fatty liver was developed and evaluated using Sprague-Dawley rat fed a methionine choline deficient (MCD) diet for 3 weeks. Rats subsequently underwent partial hepatic ischemia with reperfusion.

Results: Overall survival after reperfusion was lower in fatty liver rats ($p = 0.01$). Necrotic area of liver tissue, 8-OHdG, TNF- α , and IL-6 were higher in fatty liver rats ($p = 0.01$). p47phox was over expressed in fatty liver rat after reperfusion, and reached peak at 24 h after reperfusion. TLR4 was over expressed in fatty liver rat after reperfusion, and had two peaks at 4 and 24 h after reperfusion with release of high mobility group box 1 (HMGB1) from nucleus. Fatty liver rats with NOX inhibitor improved survival rate, necrotic area of liver tissue, 8-OHdG, TNF- α , and IL-6 ($p = 0.01$).

Conclusion: In fatty liver, compared with normal liver, ischemic reperfusion liver injury was increment accompanying with increase of HMGB1, TLR4 and NOX2. Inhibition of NOX activation from this cascade leads improvement of oxidative stress and is useful for inhibition of ischemic reperfusion injury of fatty liver.

P-0984

A rare infectious complication after living donor liver transplantation

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After liver transplantation, infections are still the most common and often life-threatening postoperative complications. Methicillin-resistant *Staphylococcus aureus* (MRSA) infection frequently complicates the postoperative course of liver transplant recipients. It has been well described that MRSA associated bacteremia, pneumonia and surgical site infection are common. But, MRSA infection manifesting as

pyogenic spondylodiscitis is very rare. To our knowledge, pyogenic spondylodiscitis due to MRSA in lumbar spine after living donor liver transplantation (LDLT) has not been previously reported. Here, we report a 50-year-old man who developed pyogenic spondylodiscitis caused by MRSA after LDLT. Our patient underwent LDLT for HBV related cirrhosis. Immunosuppressive treatment was administered with basiliximab, tacrolimus, corticosteroids and mycophenolate mofetil. He discharged on postoperative the 28th day with uncomplicated course. At 1 week after discharge the patient was readmitted for abdominal pain and high fever. Bile leakage at the anastomosis site was found by ERCP and managed successfully with endoscopic nasobiliary drainage (ENBD). The culture of drained fluid showed MRSA and he was treated with vancomycin for 4 weeks. These treatment resulted in resolution of the infection. However, one month later the patient presented with severe back pain. At this time, MRI showed massive spondylodiscitis of lumbar 2–3 spine and paraspinal abscess formation. Our patient underwent surgical debridement and primary bone graft. MRSA was cultured from the abscess. Postoperatively, the patient received intravenous vancomycin for 2 weeks and revealed complete outcome with no neurological sequelae. Presently he is followed up and doing well without rejection and other complications.

P-0985

Donor derived dengue infection in living donor liver transplantation recipient: case report

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Number of infections are transmitted from donor to recipient through organ transplant. Transplantation related transmission of dengue virus from donor to recipient is rarely reported. We report a case of transmission of dengue virus from donor to recipient after living donor liver transplantation. The recipient had history of multiple admissions for hepatic encephalopathy and ascites. He was admitted in ICU for 15 days prior to transplantation for chronic liver disease ascites and Acute Kidney Injury. Donor was admitted one day prior to transplant. Donor spiked fever on POD2 after transplant followed by thrombocytopenia and elevated liver enzymes. Donor blood test came positive for dengue NS 1 antigen. Recipient had a similar clinical picture from POD5. Recipient was also confirmed with dengue fever by positive NS1 antigen. Hence a diagnosis of post transplant donor derived allograft related transmission of dengue infection was made. Both recipient and donor were treated with supportive measures and discharged after their full recovery on POD9 and POD18 respectively. The effect of immunosuppression on dengue presentation is still unclear. In our case recipient developed dengue fever similar to general population without any feature of severe graft dysfunction. Conclusion; dengue virus can be transmitted from donor to recipient through organ transplant. In this case immunosuppression did not have any adverse effect on evolution of dengue fever in the recipient. Donor screening (In season time) for dengue virus in hyper endemic zone (Delhi) seems to be the best preventive method for donor derived transmission of dengue to recipient.

P-0986

Successful percutaneous thrombectomy for portal vein thrombosis following liver transplant

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This is the case of a 48-year-old woman with type 2 Diabetes, chronic hypertension, and HCV cirrhosis presented a Child Pugh Score of B. Patient received an orthotopic liver transplant (OLT), during the surgery a chronic portal vein thrombosis was found in the receiver, performing thrombectomy of the thrombus without complete success. During the first 48 h the patient presented elevated hepatic enzymes, refractory ascites, with an excess drain of more than 5 liters per day, and general deterioration. Due to the extension of the clot and the torpid evolution of the patient, surgical thrombectomy is performed. Nevertheless, due to the morphological characteristics and the chronicity of the thrombus, the complete extraction is not achieved. The day after the second intervention, deterioration of the renal and hepatic function with hyperamylasemia and metabolic acidosis is found. A magnetic resonance imaging shows an increase of the thrombus extension. Procoagulant factors were analyzed and an antithrombin III deficiency was found. Percutaneous thrombectomy was realized by the interventional radiologists, with no immediate complications post-procedure observed. At the end of the procedure permeable flows were corroborated with doppler ultrasound. After the procedure, anticoagulation with low molecular weight heparin is administered. After 3 weeks the patient is discharged with renal and hepatic function improvement. Actually, the patient is alive 10 months post OLT, with normal renal and hepatic function. She is being treated with tacrolimus, mofetilic acid, and oral anticoagulants. Control ultrasounds and hepatic function tests are normal.

P-0987

Successful living domino liver transplantation using liver from a maple syrup urine disease patient

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Introduction: There are 11 reports of domino liver transplantation (DLT) using maple syrup urine disease (MSUD), but structurally and functionally normal, explanted liver into a second recipient. We report the 4 patients who were successfully treated by DLT. Patients and Method: The age of 4 s recipients at DLT was median 30.5 month-old (23–243 month-old). The primary disease was protein C deficiency, biliary atresia, familial hypercholesterolemia, congenital hepatic fibrosis. We evaluated the transection site of the vessels based on 3D-CT of the first donor and recipient pre-operatively.

Result: Three whole liver grafts and 1 right lobe graft with MSUD were used for second recipients. Actual graft-recipient body weight

ratio was median 2.10 % (range 1.24 to 3.52 %). Cold ischemic time was median 4.6 h range 3.6 to 5.8 h). Warm ischemic time was median 29.5 min (range 23 to 45 min). Operation time was median 476 min (range 303 to 750 min). After living domino liver transplantation, we had no complications associated with vessels reconstruction. All patients were alive and maintained normal liver function tests and branched-chain amino acids homeostasis. The follow up periods were median 270 days (range 164 to 500 days). **Conclusion:** DLT using liver graft with MSUD could be a useful treatment option for pediatric liver diseases. It is important to evaluate the transaction site of vessels in first recipients.

P-0988

Super marginal liver graft; lacerated liver in open abdomen setting; a case report

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Nowadays, the donor scarcity still the main problem of the development of transplantation program. The imbalance between extremely increasing demand and organ supply led to extended criteria donor approach. The successful use of donors with infectious disease and septic shock has been reported. However, Organs from deceased donors with traumatic abdominal injury and open abdomen are usually discarded due to risks of severe infections. These donors are often a source of valuable organs, as they are frequently young and otherwise healthy. There is just only one case report which the liver graft from open abdomen was used successfully. Herein, We report of a case of liver transplantation using a traumatized liver allograft procured from a deceased donor with an open abdomen. The donor is a 16-year-old patient who had blunt abdominal trauma and severe head injury from car accident, resulting damage control laparotomy with suturing lacerated wound at liver and abdominal packing. Second laparotomy was performed 4 h later due to ongoing bleeding which was ended with abdominal packing again. The donor subsequently was pronounced brain death and the family consented to organ donation. A multiorgan procurement was performed and liver was transplanted to 52-year old patient who had multiple hepatocellular carcinoma. The post operative course was going well without any infection or rejection. In conclusion, the use of donor livers with preexisting trauma in open abdomen setting can be used as alternative to expand organ donor pool.

P-0989

Coexistence of gastrointestinal stromal tumor of the stomach and small bowel adenocarcinoma

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Objectives: The coexistence of gastrointestinal tumors (GISTs) with other several primary malignant neoplasms has been demonstrated.

However, there is no study that reported simultaneous occurrence of primary small bowel adenocarcinoma (SBA) and a GIST located in the stomach. Here, we report the first case of SBA and a synchronous gastric GIST discovered incidentally in a 79-year-old male patient who presented with nausea and vomiting and discuss its features in light of data obtained.

Methods: Surgical resection and clinicopathological characterization were performed for the masses identified through endoscopic and radiologic studies.

Results: Computed tomography scan showed a 13 mm mass located at the posterior wall of the gastric antrum and another mass lesion partially occluding the jejunum. A biopsy specimen was obtained from the mass obstructing the lumen at the proximal jejunum by double balloon enteroscopy. The tumor was reported as adenocarcinoma. The patient was operated by the general surgery team. The features of the submucosal lesion resected from the antrum were consistent with a diagnosis of a GIST which contained spindle cells and showed positive staining with CD-117 and CD-34. Mitosis was not observed and Ki67 index was below 10 %.

Conclusions: To our best knowledge, this is the first case of coexistence of GIST of the stomach and a SBA to be reported in English language literature. The prominent characteristics of our case include its antral localisation, male sex, absence of mitosis and incidental diagnosis.

P-0990

Endoscopic findings around gastroesophageal junction. experience from tertiary hospital in Pakistan

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Background: Gastroesophageal reflux disease (GERD) is a clinical diagnosis and upper gastrointestinal endoscopy is done to evaluate the degree of esophagitis, associated features, and complications. Assessment of the gastroesophageal junction (GEJ) during diagnostic upper gastrointestinal endoscopy (UGIE) is beneficial. The aim of this study is to determine the frequency of various endoscopic findings around gastroesophageal junction in patients with clinical diagnosis of GERD.

Subjects and methods: All patients older than 18 years and younger than 60 years of either gender presented with symptoms of GERD for more than 8 weeks were enrolled. All patients went under endoscopic examination of the esophagus, GEJ, stomach and duodenum using the same equipment. Endoscopic findings were recorded on a preformed proforma.

Results: Mean age of the patients was 43.07 ± 18.94 years. There were 60 (43.20 %) male and 79 (56.80 %) females. Mean weight of the patients was 70.91 ± 14.70 Kg. Mean duration of the symptoms was 11.44 ± 2.02 months. Endoscopic observations showed that, reflux esophagitis was found in 14 (10.10 %), hiatus hernia 19 (13.70 %) and columnar lined esophagus 34 (24.50 %) patients.

Conclusion: Endoscopic observations revealed that reflux esophagitis, hiatus hernia and columnar lined esophagus are predominant findings around gastroesophageal junction in patients with clinical diagnosis of GERD in our population. Our other observation during study was that severity and prevalence of GERD is on increasing trends in Pakistani population.

P-0991

The expression of ADIPOR1/R2 in gastric intestinal metaplasia and gastric carcinoma

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Introduction: Adiponectin (ApN) is a 244-amino acid protein mainly secreted from the adipose tissue and involved in various physiological functions. ApN exerts its metabolic effects by binding to two major receptors: adiponectin receptor-1 (Adipo-R1) and adiponectin receptor-2 (Adipo-R2) (1). Recent studies have reported ApN's involvement in the progression of cancer (2). However, there are no studies evaluating the relationship between Adipo-R1/R2 expression and gastric intestinal metaplasia (IM), which is a predisposing factor in gastric cancer (GC) development, and Helicobacter pylori (H. pylori) infection.

Aims and methods: In this study we aimed to investigate the relationship between the Adipo-R1/R2 expression and H. pylori infection in patients with GC and gastric IM. Forty patients that underwent gastric resection and 56 patients that developed gastric IM were included in the study. The Adipo-R1/R2 expression and the presence of H. pylori were examined immunohistochemically.

Results: The univariate analyses showed that the expression of Adipo-R1/R2 in GC patients was significantly lower compared to both complete metaplasia (CM) and incomplete metaplasia (ICM) patients ($p < 0.0001$ for both). According to multiple multinomial logistic regression analysis, Adipo-R1/R2 expression in the CM group was significantly higher than in the GC group ($p = 0.05$, $p = 0.014$, respectively). Moreover, Adipo-R1/R2 expression was significantly higher in ICM group compared to the GC group ($p = 0.012$, $p = 0.045$, respectively). However, in both analyses no significant difference was determined in terms of H. pylori positivity between the groups.

Conclusion: The resulting data suggests that ApN plays a role in GC processes via Adipo-R1/R2 receptors.

P-0992

Upper gastrointestinal endoscopic findings in more than 8500 patients

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The aim of the study was to evaluate esophagogastroduodenoscopy findings in patients presenting with different complaints. This retrospective, observational study was conducted in Imam Reza hospital (Tabriz-Iran) from 2006 to 2010. The endoscopies were performed as per the standard protocol with diagnosis based on accepted criteria. A

total of 8585 patients with different complaints had endoscopy. Amongst them Male 54.5 % (n = 4678) and female 45.5 % (n = 3907). The mean age of the study population was 49.12 (SD = ±18.26). UGIE findings include hiatus hernia 19.1 % (n = 1641), gastric ulcers 9.3 % (n = 802) and ulcers located on the body of the stomach had highest prevalence 45.8 %. (Table for location of gastric erosion 16.5 % (n = 1416) with a high prevalence in anterior wall of the stomach 45.3 %, duodenal ulcer was seen in 7 % (n = 599) and duodenal erosion 6.8 % (n = 581). All of the erosions and ulcers in first part of the duodenum, esophageal varies 5.3 % (n = 457). (53.2 % of the varies in f2–f3)). Esophagitis was seen in 20 % (n = 1714) the major of them states in grade A (60.6 %), Barret esophagus also was seen in 1.1 % (n = 91) (short = 80.2 and long = 19.8) stomach polyp was seen in 1.3 % (n = 110) with high prevalence in body (35.3 %), bleeding was seen in 1.2 % (n = 101) (1.1 % from esophageal and stomach, 0.1 % from first part of the duodenum).Gastro-duodenal erosion, esophagitis, duodenal ulcer disease and hiatus hernia are the commonest endoscopic findings in patients. Upper GI endoscopy is a useful diagnostic modality in elucidation of the causes of gastrointestinal problem.

P-0993

Evaluate the effects of *Bacillus coagulans* in functional bowel disorders

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Background: *Bacillus coagulans* (PROBACI) have been examined for their effectiveness in a diverse spectrum of infectious or inflammatory bowel diseases. The aim of this study is to evaluate the effects of PROBACI in various functional bowel symptoms.

Methods: Thirty-eight patients with function disorders in GI clinic were enrolled in this observational study using PROBACI (300 mg containing *Bacillus coagulans*: 1 × 10⁹ cfu) twice a day. Patients were evaluated every 2 weeks over a 4-week period using validated questionnaires and standard biochemical testing. Abdominal pain, abdominal distention and globe assessment were evaluated using a 5-point Visual Analog Scale. Gut microbiota composition of Firmicutes/Bacteroidetes ratio was analyzed by sequencing 16S ribosomal RNA genes from stool samples at the day 0, 14 and 28.

Results: Twenty-four patients were recognized as constipation dominant group and 14 patients were diarrhea dominant. Overall, the 38 patients achieved significant improvements in abdominal pain (p = 0.0009), abdominal distention (p = 0.0002) and global assessment (2.7 ± 0.6 to 3.6 ± 0.7, p = 0.0001) from day 0 to day 14. And these improvements maintained from the Day 14 to the Day 28. The constipation group achieved more improvements than diarrhea group in the feeling of defecation (2.6 ± 0.5 to 3.6 ± 0.8 vs. 2.5 ± 0.7 to 3.1 ± 0.7 p = 0.018) and normalization of defecation style (50 % vs. 7.1 %, p = 0.007) at Day 28. The Firmicutes/Bacteroidetes ratio was significantly increased in symptoms improved group (p < 0.05). **Conclusions:** *Bacillus coagulans*-based product was effective in improving various gastrointestinal symptoms in this observational study.

P-0994

Enteral parasite detection by stool exam, abdominal US/CT, and colonoscopy during health checkup

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Background: The aim of our study was to study the clinical implication of enteral parasite detection by stool exam in a single health checkup center for 10 years and to compare the detection rate of clonorchiasis and trichuriasis by organ specific exams such as abdominal ultrasonography US, CT, and colonoscopy with that by stool exam.

Methods: Data about 197,406 stool samples were collected from 99,451 subjects in a single health checkup center for 10 years. Annual detection rate of each parasite by stool exam, abdominal US/CT, and colonoscopy were analyzed.

Results: 3,472(1.73 %) stools from 3,422 subjects were positive for parasitic ova. 1,508 clonorchiasis, 959 metagonimiasis, 855 trichuriasis, 142 ascariasis, 5 trichostrongylosis, 2 taeniasis 2, and 1 enterobiasis cases were detected by stool exam in common order. Clonorchiasis was more commonly detected in first exam takers than in reexam takers and its detection rate gradually decreased over the 10 years. However, this decrease was not observed in other parasites. Colonoscopy was performed in 258(30.18 %) trichuriasis subjects, and adult worm was found in 23(8.91 %) subjects. Abdominal US or CT was performed in 1,505(99.8 %) clonorchiasis subjects, and only 37(2.46 %) subjects had images compatible with clonorchiasis.

Conclusions: Stool exam performed during health checkup may contribute to reducing the burden of silent but carcinogenic clonorchiasis. However, abdominal US, CT, or colonoscopy may not be an alternative to a stool exam for screening enteral parasites during health checkup.

P-0995

Adverse effects of sodium arsenate exposure to gestational mothers

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Aims: Naturally occurring arsenic is a ubiquitous environmental toxicant known to contaminate water supplies around the world, primarily in the form of arsenate and arsenite. Exposure to high levels of arsenic may cause carcinogenicity, mutagenicity and teratogenicity. Arsenate and arsenite are absorbed efficiently through gastrointestinal tract and distributed in organs after maximum detoxification through liver. Present study was designed to explore the teratogenicity and hepatotoxicity in developing mice.

Methodology: Different doses of sodium arsenate, 0.00, 75, 37.5 and 18.75 µg/g B.W. were administered to gravid mothers on day 6–12 of gestation, and fetuses were recovered on day 18 of gestation.

Results: Fetuses recovered, showed certain organs disproportions like micromelia and hyperextensions, hygromas, skin hemorrhage, short tail and distorted axis along with fetal resorptions. Body weight of fetuses were reduced significantly (P < 0.05) with increase in concentration of dose. Morphometrically, head circumference, eye

circumference, tail length, forelimb and hind limb size showed dose dependent decrease. A significant decrease ($P < 0.05$) was observed in weight of liver of gestated mothers at higher concentrations. At histological level necrosis of maternal liver was also noticed.

Conclusion: The observed results indicate the potential toxicity of sodium arsenate concentrations used during this study. It can be harmful to mothers at gestational stage and can cause liver toxicity.

P-0996

A case of Fanconi syndrome induced by TCM in a chronic drug induced liver disease patient

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Hepatotoxic complications of Traditional Chinese Medicine have been well described. However, Fanconi syndrome with renal tubular acidosis due to the TCM is rare. Here we report a case. A 51-year-old female was admitted to our hospital with fatigue and jaundice. She has been hospitalized for the three times with the same symptoms in our hospital. Two years prior to presentation, she started taking some traditional Chinese medicine for palpitations in a local hospital. She took the medicine for 4 months but the definite composition of medicine was unknown. During the second hospitalization in our hospital, we performed the liver biopsy. The results showed G4S3. Combined with the medical history and accessory examination, a diagnosis of chronic drug induced liver injury was made. At the same time, renal dysfunction, hypokalemia, hypophosphatemia, and hyperkalemia was presented. After ruling out the other causes of hypokalemia and renal function damage, a diagnosis of secondary Fanconi syndrome was made, and was believed to be induced by Traditional Chinese Medicine. Considering DILI and renal tubular acidosis, corticosteroids treatment was initiated. Then, all liver biochemical parameters had improved, and hypokalemia and hypophosphatemia was correct. The patient is followed up. Fanconi syndrome induced by TCM is rare. Therefore, we should pay more attention to it.

P-0997

The protective effect of Djulis and its bioactive compounds against CCl₄-induced liver injury

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The protective effect of water extracts of Djulis (*Chemopodium formosaneum*, WECF) and its bioactive compounds, rutin, kaempferol and betanin, against carbon tetrachloride-induced liver injury in rats was investigated. Rutin, kaempferol, betanin and another three compounds were identified and present in WECF using HPLC-DAD and HPLC-MS/MS analysis. Oral administration of WECF to rats at 2.5 mg/kgbw for 28 consecutive days before a single dose of CCl₄ demonstrated a significantly lowered serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels, and attenuated histopathological changes in CCl₄-treated rats. Although

WECF induced CYP2E1 activity, WECF inhibited lipid peroxidation, restored hepatic glutathione (GSH), enhanced superoxide dismutase (SOD), and reduced DNA damage in CCl₄-treated rats, which explained the antioxidant action and suppressed oxidative stress. Rutin, kaempferol and betanin at 1.0 µg/kgbw significantly restored GSH levels and reduced DNA damage in CCl₄-treated rats. In addition, betanin at 1.0 µg/kgbw increased SOD activity. Taken together, this study is the first time to demonstrate that WECF protects rat liver from CCl₄-treated liver injury due mainly to attenuating oxidative stress. The presence of bioactive compounds in WECF may partly be responsible for the hepatoprotection of WECF.

P-0998

Tuberculostatic hepatotoxicity: focused on NAT2 enzyme status and polymorphism of CYP 2E1

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Background: Antituberculous agents is one of a drug-induced liver injury's risk factors. Individual susceptibility in drug metabolism and detoxification due to genetic factors was also reported lately. Among the individual susceptibility factors, we would like to identify the genotype of N-acetyltransferase 2 enzyme status and polymorphism of cytochrome P450 2E1 as a risks factors of antituberculous agent-induced liver injury in Indonesia population.

Methods: This is a case control study in 50 tuberculous' patient at Cipto Mangunkusumo hospital, Jakarta, Indonesia and Private Pulmonologic clinics which were divided in control group (n = 25) and case group (n = 25).

Results: From 50 subjects, the baseline characteristic were mostly female (62 %), age >45 years old (56 %), normoweight (66 %). Bivariate analysis were performed to identify the risk factors and only slow acetyltransferase status of NAT2 enzymes was found significant with crude OR 1,563 (95 % CI 1,165-2,097; p = 0,002) but not in a CYP 2E1 polymorphism status.

Conclusions: Slow acetyltransferase status of NAT2 enzymes was a risk factor for antituberculous agents induced liver injury.

P-0999

Incidence of drug induced liver injury in government hospital patients on Anti-Koch's treatment

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Introduction: Tuberculosis (TB) remains a major global health problem. Anti-TB drugs have proven efficacy against TB, however, they can induce various adverse events of which hepatotoxicity is the most serious. Anti-tuberculosis drug induced liver injury (ATLI) is emerging as a significant threat to TB control, though limited data is available at present. Study aims to estimate the incidence of ATLI and understand its clinical features.

Methodology: Single center, prospective study which consisted of cohort of TB patients who received DOTS treatment at EAMC from December 2013 to May 2014. Only 285 patients who were at least

18 years of age were included. Clinical and laboratory features of ATLI were monitored for the treatment duration.

Results: We monitored 240 TB patients, 52 were dropped from the study while 188 continued. Nine patients developed ATLI with cumulative incidence of 4.8 % (95 % CI, 2.4 to 7.19 %). Nausea, abdominal pain were the most frequently observed signs and symptoms. Three (33.33 %) ATLI patients had severe hepatotoxicity, 7 (77.77 %) recovered, 1 (11.11 %) failed to respond to treatment with continued elevation of aminotransferases and 1 (11.11 %) died as a result of ATLI.

Conclusion: For this cohort, ATLI incidence was higher compared to data from China and Canada, comparable with Hongkong, and Singapore but lower than Taiwan. Presence of comorbidities showed trend to increase ATLI, however, further analysis only showed those with liver and biliary diseases to be statistically significant. Larger cohort of patients is suggested.

Keywords: Drug Induced Liver Injury, Antituberculosis Induced Liver Injury

P-1000

Clinical and histologic features of azathioprine-induced hepatotoxicity

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Background: The incidence of azathioprine-induced hepatotoxicity varies, and most studies were performed in IBD patients. This study aims to report clinical features of azathioprine-induced hepatotoxicity.

Methods: Medical records of patients received azathioprine from 2010 to 2014 were reviewed. Hepatotoxicity was defined as serum ALT or AST or total bilirubin >2 times upper limit normal. Other causes of liver diseases were excluded. All subjects were followed until resolution of liver injury.

Results: 293 patients received azathioprine were retrospectively reviewed. Common indications were rheumatologic <35 %> and hematologic diseases <15 %>. Eight patients <2.7 %> were diagnosed with azathioprine-induced hepatotoxicity. The median age was 45 years, and 6 were female. The causality score was considered to be highly probable <n = 2> and probable <n = 6>. The latency to onset of hepatotoxicity ranged from 7 to 236 days <median 70>. Four patients were symptomatic. There was a trend toward receiving concomitant corticosteroids in azathioprine-induced hepatotoxicity group <88 vs. 52 %, P = 0.05>. According to R-ratio, mixed pattern <50 %> was more frequent than cholestatic <37.5 %> and hepatocellular <12.5 %>. Liver biopsies were performed in 2 patients, and showed intrahepatic cholestasis with mild portal inflammation with increased intrahepatic eosinophils. All patients recovered fully after a median time of 41 days <range 8–77>. Two patients developed prolong cholestasis >2 months. One patient developed hepatotoxicity again after re-administration of azathioprine. None had liver failure or required liver transplantation.

Conclusion: Hepatotoxicity is relative uncommon among patients receiving azathioprine, and predominantly is mixed hepatocellular and cholestatic in nature. All patients recover fully after drug discontinuation; however severe cholestasis can occur.

P-1001

Drug-induced liver injury at a tertiary care hospital in India: etiology, presentation and outcome

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Background: Drug-induced liver injury (DILI) is rare; however, it may result in significant morbidity or death. Causes and outcome vary according to regions; acetaminophen being common in the West and complementary medicines in the Far East. We evaluated the causes, clinical features and outcome of DILI in India.

Methods: Patients with suspected DILI were enrolled based on pre-defined criteria and followed up for at least 6 months or until normalization of liver tests. We collected data from 80 individuals diagnosed with DILI at our hospital (42 men; median age, 38 years). Liver tests immediately on admission or outpatient visit were measured. Causality assessment was done by applying the Roussel Uclaf Causality Assessment Method model.

Results: DILI was caused by a single prescription medication in 30 %, by complementary and alternative medicine (CAM) in 12 %, and by multiple prescription agents in 58 % of cases. The most commonly implicated drugs were antitubercular drugs (ATD) (42 %), antiepileptic drugs (16 %), antiretroviral drugs (12 %), methotrexate (6 %), and other drugs like atorvastatin, paracetamol, etoposide, diclofenac, interferon and thalidomide (2 % each). The median duration of therapy was 31.5 days. 52 % patients had jaundice. 70 % patients were hospitalized for a median of 10 days (range, 3–45 days). Out of 42 % patients with ATD induced hepatitis, 61.9 % received ATD empirically. 4 % died due to liver disease.

Conclusions: DILI results in significant overall mortality (4 %). ATD, anticonvulsants, CAM and antiretroviral drugs are leading causes of DILI in India.

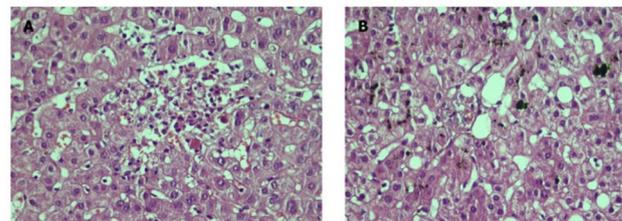


Figure A: Liver biopsy of patient #5 shows scattered hepatocyte necrosis with focal sinusoidal dilatation and intra-canalicular and intra-hepatic cholestasis

Figure B: Liver biopsy of patient #8 shows marked intra-hepatic cholestasis and bile duct injury with mild peri-portal inflammation

Patient	Age	Sex	RUCAM	Daily dose	Time to onset (days)	Pattern at onset	Peak serum ALT (U/L)	Peak serum AST (U/L)	Peak serum ALP (U/L)	Peak serum total bilirubin (mg/dl)	Time to recovery (days)
1	40	M	9	50	132	Mixed	233	73	130	8.3	63
2	43	F	8	50	85	Hepatocellular	511	419	134	0.7	63
3	40	M	6	75	236	Cholestatic	131	107	198	0.8	36
4	48	F	7	50	7	Mixed	138	211	94	0.9	21
5	72	F	9	50	48	Cholestatic	435	561	661	13.4	77
6	32	F	6	50	104	Mixed	248	77	138	0.3	8
7	50	F	7	50	55	Mixed	342	245	294	1	17
8	23	F	7	50	21	Cholestatic	366	336	1075	11.9	45

Table: Pattern of liver injury of 8 patients with azathioprine-induced hepatotoxicity

*P-1002***A rare case of levothyroxine-induced Liver Injury****Huiyu Lin, Wei Lyn Yang**

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Introduction: Drug-induced liver injury (DILI) from levothyroxine has rarely been reported. We present an idiosyncratic case of levothyroxine-induced cholestasis.

Case description: A 75-year old Chinese gentleman with significant comorbidities of ischaemic cardiomyopathy (EF 20 %, declined cardiac resynchronisation therapy) and chronic renal impairment was admitted for evaluation of confusion. Tests revealed that his delirium was due to severe hypothyroidism secondary to autoimmune hypothyroidism and he was started on levothyroxine replacement 50 mcg/daily.

His liver function tests (LFTs) checked 9 days later were cholestatic. Viral and autoimmune markers were negative. Ultrasound and Magnetic resonance cholangiopancreatography revealed fatty liver but no biliary obstruction. Fluid overload was corrected with diuresis, nonetheless cholestasis worsened over the next week.

Thorough drug history and RUCAM scoring was suggestive of DILI secondary to thyroxine. Liver biopsy was deemed unlikely to change diagnosis or management. Following discussions with the Endocrinologists, levothyroxine was discontinued. LFTs showed improvement within 2 days.

On the third day of improvement, liothyronine was started instead. His LFTs improved further over the 11 days. In view of significant cardiac history, liothyronine was deemed high risk in the long-run, thus levothyroxine was gradually reintroduced 2 weeks from date of cessation. LFTs initially worsened but improved subsequently, though the patient ultimately succumbed to pneumonia.

Discussion: Only four cases of levothyroxine causing transaminitis have been reported. This is the first case of levothyroxine inducing cholestatic LFTs. The postulated mechanisms of injury include allergy and additives to levothyroxine tablets. Liothyronine can be used as an alternative as shown in this case.

*P-1003***Atypical onset of bicalutamide-induced liver injury****Seok Hyun Kim^{1,2}, Seok Won Kim^{1,2}, Jong Seok Joo^{1,2}, Eaum Seok Lee^{1,2}, Byung Seok Lee^{1,2}**

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Androgen deprivation therapy is the mainstay of treatment for metastatic prostate cancer and is commonly used for neoadjuvant or adjuvant treatment. Bicalutamide is a non-steroidal anti-androgen frequently used during the initiation of androgen deprivation therapy along with a luteinizing hormone-releasing hormone agonist to reduce the symptoms of tumor flare in patients with metastatic prostate cancer. As side effects, bicalutamide can cause gynecomastia, fatigue, and decreased libido through competitive androgen receptor blockade. Additionally, although not as common, drug-induced liver injury has also been reported. Herein, we report a case of hepatotoxicity

secondary to bicalutamide use. Typically, bicalutamide-induced hepatotoxicity develops after a few days; however, in this case, hepatic injury occurred 5 months after treatment initiation. Based on this rare case of delayed liver injury, we recommend careful monitoring of liver function throughout bicalutamide treatment for prostate cancer.

*P-1004***Ecstasy induced steroid-responsive acute hepatitis****Tarkan Karakan¹, Zeynep G Sargin¹, Ibrahim K Onal¹, Tarkan Karakan^{1,2}, Cengiz Karacin², Betul Ogut³, Gulen Akyol³**

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Ecstasy (methylenedioxyamphetamine, MDMA) is a popular recreational drug worldwide and it has been associated with acute hepatitis and liver failure. Management is usually conservative. Here we present an MDMA induced severe hepatitis with autoimmune features that was successfully treated with steroids. A 22-year old male admitted to our department because of jaundice. One month ago he started using three to five pills of MDMA daily. His biochemical results were: total/direct bilirubin-24/20 mg/dL, alanine aminotransferase-1825 IU/L, aspartate aminotransferase-1268 IU/L, alkaline phosphatase-111 IU/L, gamma glutamyl transferase-99 IU/L, albumin-4 g/dL. His coagulation profile was normal (INR-1.1). Serum viral markers including anti HAV IgM, antiHCV and anti HBcIgM were negative. Specific antibodies: antinuclear (ANA), antimitochondrial (AMA), antismooth muscle (ASMA), anti-liver/kidney microsome-1 (LKM-1) antibodies were negative. IgG was within normal limits. There was no abnormality on hepatobiliary ultrasonography. His ceruloplasmin level was normal (36 mg/dL). Liver biopsy was performed because elevation of liver enzymes and bilirubinemia was persistent for 1 week. Histopathological examination revealed acute hepatitis. There were mild interface hepatitis and mixed portal inflammatory reaction with plasma cells as well although less than 10 %. After 1 month of corticosteroid therapy (prednisolon 40 mg oral/day) his liver enzymes and bilirubin level were completely normalized. Review of the literature on this subject shows that steroid therapy is not included in the traditional approach to cases with ecstasy induced hepatitis. Regarding the possibility of autoimmune mediated injury as in our patient early biopsy and a trial of steroid therapy may be warranted in these potentially fatal cases.

*P-1005***Non-alcoholic fatty liver disease and PCOS****Metin Basaranoglu**

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Non-alcoholic fatty liver disease (NAFLD) and Polycystic Ovary Syndrome (PCOS) are the two common metabolic disorders in clinical practice. They are seen with increased frequency in people with certain medical conditions such as obesity, type 2 diabetes mellitus

(DM), hyperlipidemia, and insulin resistance. Our objective is to compare the clinical and biochemical findings of NAFLD and PCOS. **Patients and methods:** Nine women who in productive term with well-defined NAFLD, 12 women with PCOS, and a sex and age matched community control group of 7 healthy women were included in this study.

Results: Patients with NAFLD were older than the patients with PCOS. The BMI of NAFLD patients were more than the PCOS patients. In the NAFLD group: 50 % of the patients were obese, 36 % had DM, 83 % had hyperlipidemia, 89 % of the non-diabetic NAFLD patients were insulin resistant: 5 mild, 4 moderate, and 8 severe. In the PCOS group: 33 % of the patients were obese, 17 % had impaired glucose tolerance, 58 % had hyperlipidemia, 80 % of the non-diabetic PCOS patients were insulin resistant: 5 mild and 3 moderate. When compared with the non-diabetic PCOS group, the non-diabetic NAFLD group showed an increased insulin resistance with HOMA-IR, and an increased beta-cell function.

Discussion: This study showed us that metabolic abnormalities were common in both NAFLD and PCOS, and that they were seen more frequently and severely in NAFLD group than in PCOS group

P-1006

Nonalcoholic fatty liver disease is an independent risk factor for atherosclerosis in young adult.

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Introduction: The possible cause of accelerated atherosclerosis in NAFLD may be the relationship with the MetS and its components. Our primary goal was to evaluate the relationship between NAFLD and subclinical atherosclerosis in adult patients between 20 and 40 years of age. Moreover, we aimed to investigate the changes in this association according to the presence or absence of MetS.

Method: Sixty-one male patients with biopsy-proven NAFLD and 41 healthy male volunteers were enrolled. In order to exclude any interference of confounding factors, we studied a specifically selected group with no additional cardiovascular risk. PWV, CIMT and FMD levels were measured in all patients and controls.

Results: The levels of cf-PWV were significantly higher in SS and NASH patients compared to the control group ($P < 0.001$); no significant difference was found between SS and NASH patients ($P > 0.05$). We found significantly decreased FMD levels in patients with SS and NASH compared with control subjects ($P < 0.001$). Subjects with NASH had significantly greater CIMT measurements than the SS and controls ($P = 0.026$, $P < 0.001$, respectively). Although, NAFLD patients with MetS had increased cf-PWV and CIMT and reduced FMD compared to healthy subjects ($P < 0.05$), no significant difference existed between NAFLD with MetS and NAFLD without MetS in terms of cf-PWV, CIMT and FMD ($P > 0.05$). In multivariate analysis, all of these differences remained significant even after adjusting for the confounding metabolic variables.

Conclusion: The present study showed that the presence of NAFLD leads to increased risk of endothelial dysfunction and atherosclerosis in adult male patients, independent of MetS

P-1007

Clinical features and survival of non-alcoholic fatty liver disease-related hepatocellular carcinoma

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The incidence of NALFD and NAFLD related-HCC is increasing worldwide. The current recommendation for HCC screening is only in NAFLD patients with cirrhosis. This study aimed to compare survival between NAFLD-HCC and chronic viral hepatitis (HBV or HCV)-HCC and to examine the characteristics of NAFLD-HCC patients. All cases of NALFD-related HCCs ($n = 93$) and matched cases with chronic viral hepatitis-related HCCs ($4:1$, $n = 366$) diagnosed between 2001 and 2014 were retrospectively analysed and compared at a national HCC multidisciplinary service. NAFLD-HCC patients were older (70.8 vs. 58.1 years; $p < 0.001$) and had a larger mean tumour size (64 vs. 51 mm; $p < 0.05$). One-third of NAFLD-related HCCs were detected in non-cirrhotic patients. Fewer NAFLD-HCC patients underwent HCC screening (25 % vs. 58 %; $p < 0.001$). A significant number of NAFLD-HCC patients were beyond UCSF criteria at presentation (61.3 vs. 48.6 %; $p < 0.05$). NAFLD-HCC patients were less likely to undergo liver transplantation (5.9 vs. 12.8 %; $p = 0.07$). Screen-detected NAFLD-HCC had a smaller mean tumour size than non-screen-detected NAFLD-HCC (30 vs. 78.8 mm respectively; $p < 0.05$). Higher proportion of screen-detected NAFLD-HCC were within UCSF criteria (68.2 vs. 29.7 %; $p < 0.05$). There was no survival difference between screen and non-screen-detected NAFLD-HCC (45.5 vs. 34.4 %, respectively; $p = \text{NS}$) in contrast to screen and non-screen-detected Viral-HCC (57 vs. 26.9 % respectively; $p < 0.001$). NAFLD-HCC patients were less likely to undergo liver transplantation due to older age, larger tumour size at diagnosis and less HCC screening. Patients with NAFLD-HCC in an HCC screening programme had earlier detection of HCC but this was not associated with improved patient survival.

P-1008

Comparison between NASH and ASH induced hepatocellular carcinoma (HCC)

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Background: Non-alcoholic steatohepatitis (NASH) and alcoholic steatohepatitis (ASH) have similar pathogenesis but hepatocarcinogenesis may differ. We explored differences in characteristics between NASH and ASH-related HCC.

Methods: From a prospectively maintained database of HCC since 1980 in the Department of Gastroenterology and Hepatology, Singapore General Hospital, 54 NASH and 45 ASH HCC patients were identified and their clinical, biochemical and tumor characteristics were studied.

Results: Compared to ASH-HCC, NASH-HCC group was older at diagnosis (66 ± 9 vs. 72 ± 9 years, $p < 0.001$) and less male predominant (96 % vs 65 %, $p < 0.001$). Prevalence of diabetes mellitus (78 vs 36 %, $p < 0.001$) and hypertension (80 vs. 58 %, $p < 0.001$) were significantly higher in NASH-HCC whereas lipid profile and

body mass index were comparable. Liver function tests and Child-Pugh scores were comparable. There were no differences in AFP, lesions found at diagnosis (unifocal/multifocal) or prevalence of portal vein invasion. 13.0/32.0/7.5/43.0 % of NASH-HCC and 16.0/17.0/13.3/50.0 % of ASH-HCC patients were in TNM stages 1/2/3/4 respectively. 13 % in NASH-HCC received curative resection compared to 14 % in ASH-HCC. 24 % in NASH-HCC and 29 % in ASH-HCC received loco-regional therapy (RFA/TACE/SIR). More than 50 % patients in both groups were not suitable for any active therapy. **Conclusions:** Despite differences in pathogenesis and causative etiology, liver and tumor characteristics were comparable between NASH and ASH-HCC. Most patients were diagnosed late and were not amenable to curative or loco-regional therapies. Better characterization of at-risk population of patients with NASH and ASH are urgently required to optimize screening, surveillance and management.

P-1009

Non-alcoholic fatty liver disease and hepatocellular carcinoma in Maritime South East Asians

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Background: Once a Western disease, non-alcoholic fatty liver disease (NAFLD) is increasing among Asians. We evaluated the prevalence and significance of NAFLD risk factors in Asian Americans.

Methods: This was a retrospective cohort study of 824 consecutive Asian hepatocellular carcinoma (HCC) patients seen at a U.S. medical center from 1993-2015. Studied NAFLD risk factors included BMI ≥ 23 and either hypertension (HTN), diabetes mellitus (DM), or hyperlipidemia (HLD). Cryptogenic cirrhosis excluded patients with viral, alcoholic, and autoimmune etiologies.

Results: Most patients were male (76 %) with mean age 63 years. The majority were overweight with average BMI 24.0. NAFLD risk factors were highly prevalent on presentation and increased over time (Fig. 1, $p < 0.001$), as did the prevalence of cryptogenic cirrhosis (Fig. 1). Even among viral etiologies, patients with NAFLD risk factors had higher prevalence of cirrhosis (81.7 vs 70.2 %, $p = 0.002$). Maritime Southeast Asian (MSEAs) had the highest BMI (26.3) and higher rates of DM (44.1 vs 23–35 % in other Asian subgroups, $p = 0.004$), hypertension (58.8 vs 38–55 %, $p = 0.04$), and cryptogenic cirrhosis (14.5 vs 4–10 %, $p = 0.01$). They were also more likely to be symptomatic on presentation (43.9 vs 32–58 %, $p = 0.07$), had higher BCLC scores (78.3 % were class CD compared to 52–77 %, $p = 0.001$), and had decreased 10-year survival compared to other ethnic subgroups (9 % vs 25–32 %, $p = 0.07$).

Conclusions: NAFLD risk factors are associated with cirrhosis and HCC. They are becoming more prevalent, especially among MSEAs and even in patients of viral etiologies. Targeted health interventions are needed to curb their adverse health outcomes.

P-1010

Progression and HCC in Japanese patients with NAFLD

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Background and aims: Some environmental factors and genetic variation were reported to associate with histological progression and hepatocarcinogenesis in patients with nonalcoholic fatty liver disease (NAFLD). Our aim is to determine the incidence of and risk factors including PNPLA3 rs738409 for hepatocellular carcinoma (HCC) and histological progression in Japanese patients with biopsy-proven NAFLD.

Methods: This retrospective cohort study analyzed the incidence of and risk factors for HCC in 341 patients with NAFLD. PNPLA3 rs738409 genotype was determined by allelic discrimination in 133 patients. Among them, 64 patients underwent serial liver biopsies.

Results: The PNPLA3 rs738409 genotype frequencies were CC 0.14, CG 0.46, and GG 0.40. During a median follow-up period of 5.0 years, 9 patients (2.6 %) developed HCC. The cumulative rate of HCC was 1.5 % at the end of the 5th year. Multivariate analysis identified PNPLA3 genotype (GG; hazard ratio [HR] 14.2, $p = 0.035$), and low platelet count ($< 150000/\mu\text{l}$; HR 11.6, $p = 0.036$) as predictors for the development of HCC. Of 64 patients undergoing liver biopsies, liver fibrosis was improved in 18.8 % of patients, progressed in 26.6 %, and remained stable in 54.7 %. Multivariate analysis identified no reduction of platelet count (HR 4.81; $p = 0.035$) as a predictor of improvement of liver fibrosis. Carriage of G allele was not associated with histological progression.

Conclusions: Low platelet count and PNPLA3 GG genotype were independently associated with development of HCC in Japanese NAFLD patients. We should carefully monitor platelet count to early detect HCC in NAFLD, since platelet count well reflects fibrosis progression.

P-1011

Risk and mortality of gastrointestinal hemorrhage in patients with immune thrombocytopenia

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Background: The association between immune thrombocytopenia (ITP) and gastrointestinal hemorrhage was not completely understood. The purpose of this study is to evaluate the risk of gastrointestinal hemorrhage post-hemorrhage mortality in patients with ITP.

Methods: Using the Taiwan National Health Insurance Research Database, we identified 1033 adults aged more than 18 years diagnosed with ITP in 2000–2003. Non-ITP cohort consisted of 10,330 adults randomly selected and matched by age and sex from the same dataset. Incident events of gastrointestinal hemorrhage occurring after ITP from January 1, 2000, through December 31, 2008, were ascertained from medical claims. Adjusted hazard ratios (HRs) and 95 % confidence intervals (CIs) of gastrointestinal hemorrhage associated with ITP were calculated. Another nested cohort study consisted of 27,369 patients with hospitalization due to gastrointestinal hemorrhage between January 1, 2004, and December 31, 2010. We calculated the adjusted odds ratios (ORs) and 95 % CIs of 30-day mortality after gastrointestinal hemorrhage in patients with and without ITP during admission.

Results: During 78,073 person-years of follow-up, the incidences of gastrointestinal hemorrhage for people with and without ITP were 14.5 and 5.07 per 1000 person-years, respectively ($P < 0.0001$). Compared to people without ITP, patients with ITP had increased risk of gastrointestinal hemorrhage (HR, 2.61; 95 % CI 2.05–3.32). In the nested cohort study, ITP was associated with post-hemorrhage mortality (OR 1.98; 95 % CI 1.09–3.59).

Conclusion: This study suggested that patients with ITP showed significantly higher risks of gastrointestinal hemorrhage and post-hemorrhage mortality.

P-1012

Reliability and validity of UCLA SCTC GIT 2.0 for Turkish population

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Objectives: The UCLA Scleroderma Clinical Trial Consortium Gastrointestinal Tract Instrument (UCLA-SCTCGIT) 2.0 was developed to assess systemic sclerosis (SSc) associated gastrointestinal tract (GIT) symptoms severity and its impact on patients' well-being. Our objective was to translate the UCLA-GIT 2.0 from English to Turkish and to validate it among Turkish systemic sclerosis (SSc) patients.

Methods: UCLA-GIT 2.0 was adapted into Turkish using a formal forward-backward translation method. The resulting Turkish GIT 2.0 in combination with Short Form 36 (SF-36) was administered to 97 Turkish speaking patients with SSc. We evaluated the internal consistency reliability and construct validity by exploring associations between the UCLA SCTC GIT 2.0 and SF-36 scales. Internal consistency was determined by calculating Cronbach's alpha. To determine the reliability, the questionnaire was re-administered with an interval of 2 weeks to a subgroup of patients and the intraclass-correlation coefficient (ICC) was computed. Spearman correlation coefficients between GIT scores and SF-36 were computed.

Results: Ninety seven patients with a mean age of 55.37 years and predominantly female (87.6 %) were included. Cronbach's alpha value was 0.894 for UCLA SCTC GIT 2.0 scale. Intraclass correlation coefficient score for test-retest reliability was determined, 821 ($p = 0,000$). The instrument had acceptable reliability except for the

diarrhoea scale ($\alpha = .356$). Moderate magnitude correlations were determined between total GIT score and SF-36 subscales.

Conclusions: The Turkish GIT 2.0 questionnaire showed good internal consistency, high reliability and acceptable validity.

Keywords: scleroderma, gastrointestinal tract, validity, reliability

P-1013

Sarcoidosis with liver expression

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Background: Sarcoidosis is a multisystem inflammatory disease of unknown etiology that manifests as non-caseating granulomas. Although hepatic granulomas occur in 50–65 % of patients with systemic sarcoidosis, isolated liver sarcoidosis is rare. Clinical presentation varies from asymptomatic to manifest. The diagnosis is based on a histopathological characteristic finding of liver biopsy.

Case report: We reported a case of a 41-year old woman who was admitted due to a productive cough arise about 8 months ago and associated with lateward fever and thoracic pain.

Laboratory studies detected: cholestasis, pancytopenia and elevation of angiotensin-converting enzyme. Abdominal imaging techniques showed instead liver cirrhosis, splenomegaly and ascites. The diagnosis of the hepatic sarcoidosis was confirmed by histopathological examination with liver biopsy. The patient was treated with corticosteroids; after 18 months she was free of any subjective symptoms, with improvements in biochemical and clinical examination.

Conclusion: Isolated hepatic sarcoidosis should be considered in the differential diagnosis of patients with hepatomegaly and changes in functional liver tests. Hepatic biopsy is therefore indicate because only a timely diagnosis and a proper treatment can lead to both subjective and objective improvements.

P-1014

High prevalence of Type 1 gaucher disease in idiopathic hepatosplenomegaly and/or thrombocytopenia

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Gaucher disease (GD) is a rare genetic, lysosomal storage disorder. Deficiency of glucocerebrosidase (GC) cause the accumulation of glucoceramide in the tissue. GD has 3 clinical subtypes, with 95 % of the cases are Type 1 non-neuronopathic type. In Turkey, the prevalence of the type 1 GD is not known and cannot be detected during adulthood. SM, HM and/or thrombocytopenia are the most common presenting features of GD. Therefore, we aimed to investigate presence of GD in patients with idiopathic SM-HM and/or thrombocytopenia.

Material-method: Patients older than 18 years with idiopathic SM-HM and/or thrombocytopenia who were admitted to the gastroenterology clinic between July 2014 and May 2015 were evaluated. Analysis for GC was performed using peripheral blood leukocytes in dried blood spot (DBS). $<3.2 \mu\text{mol/h}$ was considered as low β -GC activity. DBS was taken again from such patients. If the result of the

second analysis was also low, genetic mutation was investigated. Treatment was given to those who were indicated for treatment.

Results: Blood samples were drawn from totally 27 patients (19 M, 8F, mean age 37.7 years). 23 patients had mild-moderate SM and 4 had thrombocytopenia. 3 had HM-steatosis, and 1 had known lipid metabolism disorder and recurrent pancreatitis. In 9 patients (30 %) β -GC levels were found to be low (7M, 2F, mean age 39.2. Enzyme replacement therapy has been initiated in one patient.

Conclusion: In this pilot study, GD was detected in 30 % of the patients with idiopathic SM/HM/thrombocytopenia, and treatment has been initiated in 1 patient

P-1015

Human placental extract can replace the phlebotomy on treating hereditary hemochromatosis

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Background: Human hepcidin made by hepatocytes controls extracellular iron by regulating its intestinal absorption, recycling by macrophages, and release from stores. Recent studies indicate that hepcidin deficiency underlies most known forms of hereditary hemochromatosis (H.H.). In the crude material (human placenta) of Laennec[®] the presence of hepcidin m-RNA was demonstrated in our previous study. In this clinical trial, the treatment by Laennec[®] which contains hepcidin-like material could really improve H.H. without employing sequential phlebotomy—was evaluated. Case H.H. 47 years-old male patient that developed type2 diabetes mellitus had elevated serum ferritin level (10,191 ng/ml). Liver biopsy revealed remarkable iron deposition and fibrosis. Chromosomal analysis confirmed the presence of Tfr2 mutations. The infusion with Laennec[®] (672 mg/days, 3 times/w) has been done for 45 months as the substitute for the repeated phlebotomy. At the end of the treatment, the serum ferritin level was decreased to 625 ng/ml. HbA1c also improved with the same dose of insulin (8.8–6.9 %). Pleural liver biopsies revealed the remarkable improvement of both in iron deposition and fibrosis in the liver.

Conclusion: The discovery of hepcidin and its role in iron metabolism could lead to novel therapies for H.H. The placenta-derived Laennec[®] which contains hepcidin-like material actually improves iron overload of H.H. patient without utilizing sequential phlebotomy, which suggests the possibility of not only improving the prognosis of the H.H. (type 1,2,3 most common) but also curing the complications such as type2 DM, liver failure and heart failure. Laennec[®] can completely replace the continuous venesection for H.H. and may substitute other hepcidin-deficient diseases.

P-1016

The diagnosis of leishmaniasis after misdiagnosis due to hepato/splenomegaly: a review of 18 cases

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Leishmaniasis, both visceral and cutaneous, is public health problem in Turkey. The official number of visceral leishmaniasis cases (VL) is around 50 per year. In the present retrospective study, 18 VL cases diagnosed by detecting *Leishmania* amastigotes in bone marrow smears in Ege University Hospital between 2007 and 2015 were evaluated. Twelve and six patients were male and female, respectively. Their mean age was 47.44 4/–16.75 (min: 23; max: 74). All patients had fever and splenomegaly and hepatomegaly/anemia, leukopenia, pancytopenia and thrombocytopenia were observed in 17, 14, 12 and 11 patients respectively. All patients had a history of admission to several hospitals before etiological diagnosis and 14 of them had been diagnosed as hematological malignancies, urinary system infections and influenza. Four patients were sent to university hospital for further analyses for the etiology of unknown fever. There were accompanying diseases in 8 patients as cirrhosis, hematological malignancy, tuberculosis, and chronic renal failure which lead to a confusion in clinical diagnosis. Fifteen patients were treated with liposomal amphotericin B while Glucantime was administered to two patients. One patient with pulmonary tuberculosis was discharged from the hospital without treatment of VL. Four patients were died related to other causes and one patient was lost because of bleeding as a complication of VL. Although, VL has been mainly seen in childhood ages in the Mediterranean Basin, adults who have chronic systemic diseases are always at risk in endemic regions and VL should be considered in differential diagnosis.

P-1017

Clinical course and outcome of PSC/AIH overlap syndrome in children

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Aim: Primary sclerosing cholangitis/autoimmune hepatitis (PSC/AIH) overlap syndrome in childhood has been poorly defined variants of PSC. The aim of this study is to clarify PSC/AIH overlap syndrome.

Methods: The diagnosis of PSC/AIH overlap syndrome was based on: (1) the cholangiographic criteria for PSC obtained by endoscopic retrograde cholangiography (ERCP); (2) a total aggregation of international revised-scoring system for diagnosis of AIH score >10 (defining the AIH as probable); (3) liver histology with interface hepatitis, lymphocyte rosetting, confluent necrosis, plasmacyte infiltration; and (4) degree of growth retardation. Ten of PSC/AIH overlap children were retrospectively compared with 21 of PSC children presenting between 2007 and 2015 at a single center.

Results: Similarities between PSC/AIH overlap and PSC included the most clinical and biochemical parameters. The PSC/AIH overlap group significantly differed from the PSC group in the following findings: positivity of ANA (90 vs 48 %, $p < 0.05$), revised AIH score (16.5 vs 10, $p < 0.05$), advanced fibrosis ($p < 0.05$). All patients were treated with ursodeoxycholic acid (UDCA). Eight patients with PSC/AIH overlap syndrome received immunosuppressants, but demonstrated no response. Immunosuppressive therapy improved biochemical markers; however, histological findings were observed to progress in all patients. Three of 10 patients with PSC/AIH developed cirrhosis, and are planned for cadaveric liver transplantation.

Conclusions: Immunosuppressive treatment for PSC/AIH cannot prevent progression toward cirrhosis. Patients with PSC/AIH overlap may have worse prognosis than patients with PSC. Further study is needed to establish the appropriate criteria for diagnosis and treatment of PSC/AIH overlap.

P-1018

Efficacy and safety of INF combined with LAM in treatment of cirrhotic children with hepatitis B

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Background: To evaluate the efficacy and safety of interferon (IFN) combined with lamivudine (LAM) in treatment of cirrhotic children with HBeAg-positive compensated hepatitis B. Methods Twenty-six enrolled patients received combination treatment with IFN (3–6 MU/m², once on alternate days) and LAM [3 mg/(kg days)] for 48 weeks. The data of HBV serological markers, HBV DNA, biochemical parameters and adverse reactions were collected at baseline and 12, 24, 36 and 48 weeks of treatment. Results At 12, 24, 36 and 48 weeks of treatment, the rates of HBeAg loss were 30.8, 42.3, 53.8 and 53.8 %, the rates of HBeAg seroconversion were 23.1, 34.6, 46.2 and 46.2 %, the rates of HBsAg loss were 0, 11.5, 19.2 and 19.2 %, the rates of HBsAg seroconversion were 0, 3.8, 19.2 and 19.2 %, the rates of serum undetectable HBV DNA were 38.5, 53.8, 73.1 and 84.6 %, and the rates of ALT normalization were 88.5, 92.3, 92.3 and 96.2 %. As the treatment duration prolonged, there was an increase in the rates of HBeAg loss/ seroconversion, HBsAg loss/ seroconversion, undetectable HBV DNA and ALT normalization. No obviously intolerable adverse reaction was found. Conclusion Combination treatment with IFN and LAM in treatment of cirrhotic children with HBeAg-positive compensated hepatitis B is effective and safe.

P-1019

Lower mitochondrial DNA copy number is associated with telomere length in biliary atresia

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Mitochondrial dysfunction has been shown to be involved in the pathophysiology of biliary atresia (BA); however, the mechanism has yet to be elucidated. There has been no study regarding the possible relationship between telomere and mitochondrial function in BA patients. This study aimed to determine the association between

mitochondrial DNA (mtDNA) copy number and telomere length in peripheral blood leukocytes of BA patients. A total of 228 subjects (114 BA patients and 114 age-matched healthy controls) were enrolled in this case-control study. In addition, two pairs of monozygotic twins with BA discordance (one set of whom suffered from BA) were also recruited. The leukocyte mitochondrial DNA copy number and telomere length were measured using a quantitative real-time polymerase chain reaction. Our findings demonstrated that leukocyte mtDNA copy number was significantly reduced in BA patients compared with healthy controls ($p = 0.0015$). Moreover, the twins diagnosed with BA had a lower mtDNA copy number than the healthy twins. Further analysis showed that leukocyte mtDNA copy number was inversely associated with telomere length in BA patients ($r = -0.49$, $p < 0.0001$). Additionally, leukocyte mtDNA copy number in BA children revealed a negative correlation with age ($r = -0.32$, $p = 0.001$). These findings suggested that leukocyte mtDNA copy number was markedly lower in BA patients than the age-matched controls. Furthermore, leukocyte mtDNA copy number was significantly associated with leukocyte telomere length in BA. The prognostic role of mtDNA copy number in BA remains to be established in a longitudinal study.

P-1020

Transfusion related acute lung injury (TRALI) due to iatrogenic intra-abdominal bleeding

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Introduction: Transfusion Related Acute Lung Injury (TRALI) can be defined as acute respiratory failure of no other cause, developing within the 6 h of transfusion of blood products. Canadian Consensus Conference and National Heart, Lung, and Blood Institute (NHLBI) working group criteria are used for the diagnosis.

Case report: A 59 year old male was hospitalized for chronic diarrhea. He was using warfarin for prosthetic mitral valve. Abdominal ultrasound showed the presence of ascites and diagnostic paracentesis was performed. Iatrogenic intra-abdominal bleeding developed after the paracentesis and patient was admitted to the intensive care unit. His physical examination was unremarkable on admission without any sign of respiratory failure. Laboratory studies were as follows: Hb: 6.5 gr/dL, Hct: 19.1, PTZ(INR):2.5. A total of 12 units of erythrocyte suspension and fresh frozen plasma (6 units each) were transfused within 48 h of admission. One hour after the institution of last transfusion dyspnea, tachypnea, tachycardia and hypotension developed. Arterial blood gases revealed hypoxia ($pO_2:65$) and hypercarbia ($pCO_2:54$). Transfusion was stopped immediately and the patient was intubated. Bilateral fine crackles were audible on lung auscultation. Chest X-ray and computerized tomography revealed bilateral pulmonary infiltrations consistent with acute pulmonary edema. After exclusion of other possible causes (hypervolemia, cardiac and infectious causes etc) TRALI was diagnosed. Clinic, laboratory and radiologic findings resolved rapidly with conservative management.

Discussion: Although TRALI is not so rare, it is usually misdiagnosed due to unawareness of the clinicians. In patients with acute lung injury, after all other causes are excluded TRALI should also be kept in mind if transfusion was done within the last 6 h.

P-1021

A case of hepatitis due to hypereosinophilic syndrome

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A 63-year-old man was referred to our hospital and admitted with a low fever, general fatigue, epigastralgia, and an itching sensation. White blood cell count and eosinophil (33200 and 26568/ μ L) and aminotransferase levels (101 and 158 IU/L) were observed to be elevated. No viral markers, antinuclear or anti-mitochondrial antibodies, parasite eggs, or anti-parasitic antibodies were detected. He had no history of drug use or allergies. The results of a bone marrow puncture examination ruled out eosinophilia caused by myeloproliferative disease. Abdominal ultrasonography, dynamic computed tomography (CT), and magnetic resonance cholangiopancreatography examinations revealed no obstruction of the bile ducts, hepatomegaly, or periportal edema. Fluorodeoxyglucose (FDG)-positron emission tomography/CT showed a slightly diffuse up-take of FDG only in the liver (mean SUV max 3.0). After the patient's liver function test results showed no improvement in response to conservative supportive care. A liver biopsy revealed hepatitis with eosinophilic infiltration. Steroid pulse therapy (methylprednisolone, 1000 mg/day) was initiated and continued for 3 days. Thereafter prednisolone was administered with a tapering protocol. The elevated levels of aminotransferase and eosinophils slowly improved. A second biopsy, performed 6 months later, showed the improvement of the eosinophilic infiltration. The patient was diagnosed with eosinophilic hepatitis due to the presence of hypereosinophilic syndrome without the dysfunction of other organs. The present case is thought to be rare, however, we should keep in mind that hypereosinophilic syndrome causes chronic liver injury.

P-1022

Some clinical associations of thrombocytopenia in liver disease of different stages and etiologies

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The purpose of this study was to define prevalence of thrombocytopenia (TCP) in chronic liver disease dependent on etiology, activity, stage and complications. In total 499 patients with mean age of 47.2 ± 13.9 years were examined. 177 of them were diagnosed with chronic hepatitis and 322—with liver cirrhosis (LC). TCP (PLT count less than 150,000/ μ L) was revealed in 59.3 % as a whole, more commonly—in LC (72.3 %) especially in the end stage liver disease (96.2 %). Prevalence of TCP was 69.5 % in viral, 61.1 %—in alcohol related etiology of liver disease, 60 %—in NASH, 55.6 %—in overlap syndrome (AIH + PBC), 46.2 %—in AIH and 22.2 %—in PBC. Strong correlation between TCP and viral etiology ($\chi^2 = 15.789^a$, $p = 0.001$), especially HBV ($\chi^2 = 15.845^a$, $p = 0.001$) and HDV ($\chi^2 = 9.872^a$, $p = 0.02$) were noticed. TCP was

also found to correlate with spleen size ($\chi^2 = 35.355^a$, $p = 0.0001$, Kruskal-Wallis H test = 41.78, $p < 0.01$), splenic (H = 8.96, $p = 0.03$) and portal vein diameter (H = 23.25, $p < 0.01$), AST activity (H = 8.08, $p = 0.04$), serum albumin level (H = 23.3, $p < 0.01$), INR (H = 11.14, $p = 0.01$), esophageal varices grade ($\chi^2 = 22.508^a$, $p = 0.007$), variceal bleeding episodes ($\chi^2 = 8.119^a$, $p = 0.044$), and hepatic encephalopathy grade ($\chi^2 = 48.106^a$, $p = 0.0001$). Thus TCP is more commonly seen in liver diseases of viral etiology (especially HBV and HDV), correlates with severity (LC), biochemical activity (AST), degree of hepatic impairment (albumin, INR), portal hypertension (spleen size, splenic and portal vein diameter, esophageal varices), and some LC complications (variceal bleeding and hepatic encephalopathy).

P-1023

Curative effect on Foscavir treatment for patients infected by HIV merger EB Virus

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Objective: Evaluation on curative effect of Foscavir treatment for EB Virus infection.

Methods: 30 patients infected by HIV merger EB Virus were randomly equally divided into Ganciclovir group and Foscavir group. The course of treatment is 3 weeks. We tested all patients' EBVDNA on 1st, 2nd, 3rd week and 1 week after treatment respectively.

Results: There is no statistical significance of the difference between two groups of patients on age, gender and CD4 lymphocyte count, HIVRNA, EBVDNA before treatment. The negative conversion ratios of EBVDNA for Ganciclovir group and Foscavir group, on the 1st, 2nd, 3rd week and 1 week after treatment, are 1/15 and 0/15, 3/15 and 6/15, 4/15 and 10/15, 5/15 and 11/15, and Chi-square values and P values are 1.034 and 0.309, 1.429 and 0.232, 4.821 and 0.028, 4.821 and 0.028, respectively. In Ganciclovir group, one patient had adverse symptoms, such as dizziness, nausea, rashes and mild decline of leucocyte. In foscavir group, one patient had adverse symptoms of headache, abdominal distension, and another patient had Mild elevation of creatinine. All the symptoms are tolerated and course of treatment is completed. There is no statistically significant difference between two groups with adverse reactions.

Conclusion: Both security and curative effect of Foscavir treatment on HIV merger EB virus are good, it is worthy of clinical promotion.

P-1024

Liver injury due to Epstein barr virus (EBV)

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Background: EBV has an important and multifaceted role in liver pathology. Liver injuries are ranging from asymptomatic elevation of liver enzymes to fulminant (rare) hepatitis failure and cirrhosis. **AIM:** To indentify the demographics, presenting features of EBV hepatitis. **Methods and materials:** We have studied 243 patients with acute hepatitis, 55 (22 ± 2.7 %) were diagnosed EBV hepatitis. The mean age was 19.5 ± 1.1 , 31 (56 %) were men. The diagnosis of EBV is exclusion with initial suspicion based on circumstantial evidence:

markers of viral hepatitis, autoimmune hepatitis and other reasons. Peripheral and biochemical blood tests as well as instrumental investigation of internal organs were carried out.

Results: Out of 55 patients 6 (11 %) had jaundice with elevated level of serum bilirubin: median 120 $\mu\text{mol/L}$ (40–200). In all the 55 patients hepatocellular injury was diagnosed with elevation of aminotransferase levels as a predominant syndrome. The median ALT level was 725 IU/L (200–1250). EBV-DNA was found in 21 (40 %) patients, EBV IgM in 48 (87 %). Ascites was found in 5 (9 %). Splenomegaly, leukocytosis $>10,000$ were common in all patients, whereas other cardinal symptoms such as lymphadenopathy, tonsillitis and high level of atypical monocytes were not common in patients with EBV hepatitis. In 52 (96 %) patients self-limited hepatitis was reported, whereas in 2 (4 %) chronic EBV hepatitis was diagnosed.

Conclusions: EBV hepatitis was 22 % among the patients with acute hepatitis. EBV should be considered as a possible causative agent for viral liver injury. EBV hepatitis is not complication. It may be unique manifestation of EBV infection

P-1025

A case of severe hemophagocytic syndrome secondary to chronic active EBV infection

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Severe CAEBV infection that has rather severe manifestations and poor prognosis with many life-threatening complications, such as EBV-associated malignant lymphoma and hemophagocytic syndrome. The following case illustrates the rapid development of severe hemophagocytic syndrome during chronic active EBV infection in a 13 year old boy who presented with intermittent fever, hepatosplenomegaly, abnormal liver enzymes and jaundice. Serological findings were suggestive of CAEBV, which was confirmed by the high circulating EBV viral load. The results of Bone marrow showed that bone marrow hyperplasia was active, the particles were increased and the phagocytic cells were easy to see. The liver function deteriorated gradually. A diagnosis of severe hemophagocytic syndrome secondary to chronic active EBV infection was made following an extensive investigation. Although we performed Artificial Liver Support Therapy and hemofiltration treatment combined with antiviral, glucocorticoid, hepatoprotective and supportive treatment, the patient died secondary to rapidly progressive hemophagocytic syndrome. We presume that hepatic involvement of CAEBV should be considered as differential diagnosis in cases showing liver dysfunction. Early diagnosis and early treatment, may improve the survival rate.

P-1026

Herpes simplex virus hepatitis: identification of institutional cases

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Herpes Simplex Virus Hepatitis (HSV) hepatitis is uncommon and underdiagnosed. It presents with fever, abnormal liver function tests

and flu-like symptoms. We identified 15 cases of HSV hepatitis in our institution from 2009 to 2014 (age range 1 month to 53 years) of which over half (53 %) were immunocompetent, 2 were pregnant and 5 (33 %) were immunocompromised. 40 % of the patients were above the age of 40 with a female preponderance of 2:1. The main features were fever (46 %), nausea (33 %), flu-like symptoms (20 %), muco-cutaneous lesions (13 %) and diarrhoea (13 %). No one presented with coagulopathy or encephalopathy. Most cases were diagnosed by serology (HSV Ig M positive) while others were proven on liver histology. Other potential hepatobiliary causes were excluded. 5 patients were treated with acyclovir based on early clinical suspicion. One patient died as a result of subacute liver failure and one pregnant patient had a miscarriage in the third trimester. In conclusion, HSV hepatitis need to be highly suspected in patients with fever, elevated liver enzymes, especially in immunocompromised patients to avoid delay in diagnosis and treatment. Early intervention with acyclovir reduces mortality as untreated, potentially, can progress to fulminant liver failure.

P-1027

Liver involvement in leptospirosis patients

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Introduction: Leptospirosis is an important zoonotic diseases which mortality rates vary between 3 and 54 %. Aim of this study is to detect the liver involvement and its impact on mortality in the course of disease. Method: The study was conducted at the School of Medicine, Karadeniz Technical University, between 01 Jan 1999–31 Dec 2014. Eighty patients with diagnosed leptospirosis were evaluated retrospectively. Leptospirosis was diagnosed by positivity of microscopic agglutination test. Hepatic involvement was compared with surviving (group 1) and mortal (group 2) patients.

Results: Mean age was 47.3 ± 17.5 (17–87) in cases. Fifty six (70 %) of them were male and 24 (30 %) female. Fever was the most common symptom in patients (90 %), nausea-vomiting the second (82.5 %), myalgia third (66.2 %). According to the ultrasound findings, hepatomegaly was detected in 20 (40 %) patients as totally. The mortality rate was 9/80 (11.2 %) in our patients. Mean AST levels were 140 ± 165 U/L in G1; 243 ± 179 U/L in G2 ($p = 0.023$). Mean ALT levels were 109 ± 144 U/L in G1, 141 ± 102 U/L in G2 ($p = 0.038$). Mean totally bilirubin levels were 10.5 ± 10.8 mg/dL in G1, 14.6 ± 8.8 mg/dL in G2 ($p = 0.026$). Mean direct bilirubin levels were 8.6 ± 9.3 mg/dL in G1, 12.2 ± 9.1 mg/dL in G2 ($p = 0.044$). There were no significant difference between ALP and GGT levels of two groups ($p = 0.728$ and 0.076 , respectively).

Conclusion: Liver involvement is common finding in leptospirosis and not only bilirubin but also liver function tests' rising may be associated with mortality.

P-1028

Chronic liver disease and crimean congo hemorrhagic fever: two overlapping disorder

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Background: More than 9700 cases of Crimean Congo Hemorrhagic Fever (CCHF) have been reported between 2002 and 2015 in Turkey with a mortality rate at 4.8 %. CCHF is characterized by fever and hemorrhage. Thrombocytopenia, elevated levels of aminotransferases, prolonged international normalized ratio (INR) and activated partial thromboplastin time test (aPTT) are frequently seen and may also indicate hepatitis. The aim of the study is to investigate the prevalence of chronic liver disease (CLD) in patients with confirmed or suspected CCHF.

Material and methods: Demographic, epidemiologic, clinical features and laboratory parameters of the patients that were admitted to Ondokuz Mayıs University Hospital with presumed diagnosis of CCHF in 2002 to 2015 were evaluated retrospectively. Relevant data of the cases with CLD were recorded.

Results: 324 of 440 patients had CCHF that confirmed by real-time polymerase chain reaction (PCR). 7 (2 %) of confirmed cases had hepatitis (Chronic Hepatitis B (n = 5), chronic Hepatitis C (n = 1), cryptogenic liver cirrhosis (n = 1)). 16 (13.8 %) out of 116 suspected cases were diagnosed as CLD (Chronic Hepatitis B (n = 3), cryptogenic liver cirrhosis (n = 4), toxic hepatitis (n = 4), unidentified transaminase elevation (n = 5)). There was no mortality in cases with CLD.

Conclusion: Chronic liver diseases should be considered in the differential diagnosis of viral hemorrhagic fevers particularly in endemic regions. Also it should be considered that these two diseases might overlap. Despite the resolution of the clinical and laboratory findings related to CCHF, in the case of persistently abnormal liver tests the patient should be investigated for underlying viral hepatitis.

P-1029

Dengue hepatological manifestation: from acute hepatitis to Fulminant hepatic failure

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Background and aims: Dengue is a common arboviral infection of tropics and sub tropics with protean disease manifestation and multisystem involvement. In India we are observing varied spectrum from mild hepatitis to fulminant hepatic failure. The aim of this study was to determine the clinical and biochemical profile of Dengue hepatitis.

Materials and methods: Retrospective study of 55 pts from two centres. All the pts had liver involvement in the form of elevated transaminases, jaundice and deranged prothrombin time.

Results: Age group of 16–60 years. Mean platelet count ~46000. Fever in all pts, pain abdomen 30(54.5 %), nausea and vomiting 23(41.8 %), melena 12(21.8 %) and encephalopathy in 4 patients. Clinical jaundice 9(14.5 %), significant ascites in 8(12.5 %), hepatomegaly in 31. Jaundice was mostly mild. Serum transaminases elevated in all. ALT two to five times elevated in 26(47.2 %) and more than 5 times in 14(25.4 %). AST was more than ALT in more than two-third. Prothrombin time abnormal more than 5 s in 9(18.8 %)

Conclusion: Dengue hepatitis presents with fever, abdominal pain, thrombocytopenia, elevated transaminases to variable extent and mainly preferential AST rise in majority. FHF and even mortality may occur in severe dengue hepatitis

P-1030

Hemophilia patients' level of knowledge of about viral hepatitis

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Objective: The aim of this study was to evaluate hemophilia patients' (HP) knowledge on viral hepatitis and to test whether their knowledge could be improved by a 2-h training programme.

Methods: The study and the training were conducted on June 6, 2015 during a full-day educational programme on different topics for HP. Patients filled up pre-test questionnaires, followed by a training given by an expert. The same questionnaire was distributed as a post-test after the training session. The questionnaire included six questions on socio-demographic characteristics, eight true/false type questions on knowledge about hepatitis and questions evaluating their vaccination status. Pre/post-tests were compared with the McNemar test.

Results: The mean age of the participating 24 patients was 37.0 ± 13.0 (min.20–max.67). Their mean duration since the diagnosis of hemophilia was 32.7 ± 12.8 (6–60) years. Among the participants, four were hepatitis B patients, two had hepatitis C and the rest were unaware of their viral hepatitis status. Before the training, 19 patients had heard of hepatitis B, 17 had heard of hepatitis A and C, four had heard hepatitis D and two had heard hepatitis E. Only two patients knew hepatitis A's routes of transmission correctly. There were more misconceptions on the routes of transmission of hepatitis B and C. After the training, their information on hepatitis A's food-borne transmission, hepatitis C's sexual transmission, the presence of a vaccine to prevent hepatitis A and the absence of a vaccine against hepatitis C improved significantly. Although 19 patients were aware of hepatitis B vaccine and 13 of hepatitis A vaccine at pre-test, only seven were vaccinated against hepatitis B and two against hepatitis A. **Conclusion:** Patient education programmes targeting special groups like haemophilia patients could both increase their knowledge and enable the detection and prevention of overlooked patients.

P-1031

A case hepatic rupture in acute fatty liver of pregnancy

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Introduction: Hepatic rupture is a potentially fatal complication of HELLP syndrome and uncommonly acute fatty liver of pregnancy (AFLP) that can be difficult to recognise in an already sick patient. We report a rare case of post-partum presentation of AFLP complicated by hepatic rupture.

Case description: A 25-year-old Filipino lady presented with full term labour. Antenatal screening tests were unremarkable. Within hours of an uncomplicated vaginal delivery, she developed severe epigastric pain and pre-eclampsia.

Investigations revealed raised bilirubin, transaminitis, renal impairment, leukocytosis; ultrasound reported hepatomegaly and moderate ascites, fulfilling Swansea criteria for AFLP.

20 hours from initial presentation, she became tachycardic, abdominal pain worsened, and haemoglobin dropped to 5.9 g/dL. Abdominal computed tomography revealed extravasation of blood into hepatic capsule, but urgent radiologically-guided angiogram did not detect a bleeding point. She required 7 pints of packed-cell transfusions over 24 h however haemoglobin remained low at 5.6 g/dL. A second angiogram showed increased amount of haemoperitoneum and size of the perihepatic haematoma, raising the suspicion of venous bleeding. Surgical opinion was sought; but she subsequently improved with supportive management within 48 h of delivery.

Discussion and conclusion: Post-partum hepatic rupture in AFLP is very rare but dangerous, thus early identification in the setting of worsening abdominal distension and acute haemoglobin drop is important. A low threshold for contrasted-scans aids in timely diagnosis; though this should be balanced against the risk of renal impairment. Early diagnosis, aggressive resuscitation and prompt consideration of radiological angio-embolization or surgical intervention are critical in this condition.

P-1032

Diagnostic value of tests commonly used in the diagnosis of intrahepatic cholestasis of pregnancy

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Total serum bile acids (TBA) levels are currently regarded as the gold standard for diagnosis of intrahepatic cholestasis of pregnancy (ICP). However, the level of TBA can fluctuate depending on the fasting state or pregnancy time and an asymptomatic elevation of TBA can be in approximately 10 % of pregnant women. Transaminases are not specific and may be normal in up to 30 % of cases. AIM: To assess diagnostic value of laboratory tests used for diagnosis of ICP and to determine more reliable cut-off value of transaminases.

Methods: Sixty six patients with ICP and 29 healthy pregnant women were included in the retrospective analysis.

Results: Comparison of the area under the curve (AUC) of all biochemical parameters showed a superior diagnostic accuracy of alanine transaminase 0.966, asparagine transaminase 0.981, direct bilirubin 0.922, TBA 0.754, cholic acid (CA) 0.789. ICP patients had significantly higher TBA levels than healthy women (32 vs 6) due to increase in CA and chenodeoxycholic acid (CDCA). CA/CDCA ratio was significantly higher in ICP patients. No significant difference was found between sensitivity, specificity, positive and negative predictive value of serum TBA, CA and CDCA. Lower cut-off values for transaminases had the similar sensitivity and specificity as the reference ranges currently used.

Conclusion: no single biomarker had higher sensitivity and specificity for diagnosis of ICP. Lower limits for transaminases cut-off values could increase the effectiveness of case finding for ICP. Only comprehensive clinical assessment may help clinicians to provide a more accurate tool for diagnosis of ICP.

P-1033

Obstetric complications in obese pregnant women, associated with liver steatosis.

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The aim of the present study was to identify the liver steatosis in obese pregnant women and to reduce the obstetric complications.

Materials and methods: The study included 203 obese women. The 132 patients were associated with liver steatosis and group for comparison—71 obese women without liver steatosis. The examination included biochemical indexes of liver function, lipid spectrum and ultrasound of the liver. Patients who were examined before pregnancy were most valuably treated in two stages during 1–3 months: Vitamin factor of group B, containing inosit (1000 mgs) and folacin (0,1 mgs) per a day and omega 3 polyunsaturated fat acids (300 mgs) per a day. Then during pregnancy was administered 3-sn Phosphatidylcholine (1800 mg per a day) for 1–3 months. Control of the liver condition was carried out three times during pregnancy.

It is shown that in pregnant women are not receiving treatment steatohepatitis were significantly more frequent obstetric complications: preeclampsia, chronic placental insufficiency, than those who got treatment. The observation showed that gestational diabetes on insulin significantly more frequent associated women with liver steatosis (50 %, p-0.001), than women just with obesity. The observation showed that gestational diabetes on insulin significantly more frequent associated women with liver steatosis (p-0.001), than women just with obesity. Conducted through the treatment, despite the initial changes of biochemical parameters of liver function, lipid spectrum, it was achieved normalization of all indexes in patients with liver steatosis. The treatment favorably affected pregnancy and delivery outcomes.

P-1034

Spectrum of liver disease during pregnancy: a cross sectional study from Pakistan

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Background: Deranged liver function during pregnancy is a common problem that could lead to adverse maternal or fetal outcomes. Unfortunately the information about spectrum of liver diseases during pregnancy in Pakistan is quite limited. Therefore the aim of this study is to determine the maternal and fetal outcomes associated with liver diseases during pregnancy and identify the factors associated with complications in these conditions.

Methodology: This was a single center, retrospective cross sectional study conducted at the Aga Khan University, Karachi. Information was collected regarding demographic characteristics, parity,

symptoms at presentation, etiology of liver disorders, mode of delivery, laboratory parameters and fetal, maternal and neonatal outcome from the medical records of all pregnant women presenting with liver disease from January 2000 to date. Data analysis was done using SPSS Version 19.

Results: The mean age of our study population was 29 ± 2.5 years. GDM was found to be the most common co-morbidity present in 14.1 % of the sample. Abdominal pain was the frequent presenting symptom in 27.5 % of the sample. Obstetric cholestasis was the most prevalent liver disease in pregnancy (40.4 %), followed by Chronic Hepatitis C (19.6 %) and acute hepatitis E (15.7 %). 17 miscarriages occurred. Beside preterm birth (18.4 %) and Low birth weight (13.7 %) as most common fetal complications, 17 miscarriages occurred. The most common maternal complications were postpartum hemorrhage (12.5 %) and preterm labor (5.9 %).

Conclusion: Liver diseases during pregnancy are associated with high fetal and neonatal morbidity and mortality. Early diagnosis and treatment may improve outcome.

P-1035

Horizontal transmission of HBV after successful MTCT prevention: a case report

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Introduction: There is few report about horizontal transmission of HBV after successful MTCT prevention. To evaluate the efficacy and safety of telbivudine in preventing MTCT of HBV in HBsAg-positive pregnant women with high viral load, we investigated 450 patients through 8 years in an open-label study. We present the case of an infant of one participant enrolled in our clinical trial with negative HBsAg at 6 months who turned out to be HBsAg positive at 3 years old.

Case presentation: A 27-year-old Chinese pregnant woman was enrolled in the trial, whose serum HBsAg and HBeAg was strong positive. Maternal viral load decreased from $2.71E + 7$ IU/ml at baseline to $3.23E + 4$ IU/ml at Week 33. The HBV DNA levels just before delivery failed to test. At Week 39 + 2, she chose to have spontaneous delivery and finally withdrawn LdT after delivery. After birth this infant was inoculated with recombinant HBV vaccine and hepatitis B immune globulin (HBIG) according to standard immunoprophylaxis procedure and feed with formula. At 6 and 8 months of age HBsAg and HBsAb of the infant was still negative. But he became HBsAg-positive in low level (9.2 IU/ml) at 3-year-old. It was noticed that this child has the habit of using his mother's toothbrush.

Conclusion: Horizontal transmission and infection can happened occasionally, particularly in vaccination failure child. Clinicians should be aware that non-responders to HBV vaccination may be susceptible to HBV infection.

P-1036

Change pattern of HBV B/C in vertical transmission

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Evolution patterns of HBV QS between genotype B and C during vertical transmission are not well understood. In this study, we enrolled ten HBV infected mother-infant pairs (4 pairs with genotype B, 4 pairs with genotype C, and 2 with co-infection) without anti-viral therapy. Serum HBV DNA of mothers and infants were sequenced, HBV QS complexity and diversity were analyzed, polymorphisms and mutation sites were recorded, and phylogenetic trees were performed. Our result showed that the QS complexities in P (amino acid), C/PreC (amino acid) and PreS1 (nucleotide) gene were significantly higher in mothers than in infants in pairs with genotype C ($p < 0.05$), however, full-length and other genes showed non-significant differences ($P > 0.05$). Unlike genotype C, QS complexity of P gene (nucleotide) was significantly higher in infants than in mothers ($p < 0.05$) in pairs with genotype B, similarly, QS complexities of full-length and other genes (except Pre S2) were also higher in infants than in mothers but without significant differences ($P > 0.05$). QS diversities of full-length and most genes in genotype B were comparable between mothers and their infants ($P > 0.05$), in pairs with genotype C, dS of P, X, RT genes, genetic distance of Pre S1 gene (amino acid) and dN of Pre S1 gene were significant higher in mothers than in infants ($p < 0.05$). Several HBV mutations correlated with immune escape, e antigen loss and drug resistance were observed in infants. The results indicated that differences of HBV QS evolution patterns between genotype B and C during vertical transmission might contribute to distinct prognosis.

P-1037

Evaluating the HAV seroprevalence of health care workers

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Aim: In recent years, studies suggest that several countries could be in epidemiological transitions due to the evident socio-economic development. Similarly in our country, the age of HAV exposure is shifting into adolescence and young adult period. The aim of the study to evaluate the healthcare workers HAV infection status.

Method: The recordings regarding the HAV exposure of health care workers who working as a cleaning person at Izmir Bozyaka Education and Research Hospital have been taken from the documents of Infection Control Committee.

Results: The recordings of 213 health care workers aged between 18 and 48 were examined. The number of persons having no HAV examination is 163(76.5 %). In total 23 (49 %) of 47 health care providers having HAV examination were defined to HAV IgG positive and 27 (51 %) of them defined to HAV IgG negative. Seronegative health care workers age groups were found six persons in 18-25 years group, eight persons in 26-30 years group and 13 persons in 31 years and older.

Discussion: Acute HAV infection may be more complicated as the person gets older and our country is still endemic in terms of HAV infection and the virus circulation is continuing widely. In our study, it has been determined that most of the healthcare workers has not been tested for HAV infection. Among the tested persons seronegativity in young adults is distinctively high. For this reason, it would be appropriate to examine the health care providers and vaccinate the ones with seronegativity.

P-1038

Evaluation of needle stick and sharp injuries among healthcare personnel

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Objective: Needle stick and sharps injuries are occupational hazards for healthcare workers. The aim of this study was to investigate the frequency of needle stick and sharp injuries among the healthcare personnel and evaluate hepatitis B and hepatitis C transmission via occupational injuries.

Method: This cross-sectional study was conducted during 2013-2015 in Izmir Bozyaka Research and Education Hospital. The records of needle stick and sharp injuries were evaluated for three years period.

Findings: Most needle stick and sharp instrument injuries had occurred among the services and intensive care unit nurses. It was found that the main cause of injury was syringe needles and needle recapping. Other injured persons were cleaning staff and trainee students. Most of the healthcare personnel has been tested and vaccinated for HBV. Any HBV or HCV infection has not been detected among the injured healthcare workers during this period.

Conclusion: The prevalence of sharp injuries is high among nurses, and also students. However more than a half of the healthcare workers with needle stick or sharp instrument injury had ignored follow-up due to their work load. Therefore repeated education programs should be given the healthcare persons, and all of the persons should be tested and if necessary vaccinated for HBV.

P-1039

Needle stick injury: an alarming problem for healthcare in Bangladesh

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Introduction: Needle stick injury (NSI) is a neglected problem in Bangladesh. Many NSI are unreported or no measures taken. This may lead to unwanted chronic hepatitis B or C, even HIV infection.

Methodology: A screening program for hepatitis C was done in the Department of Hepatology, Sir Salimullah Medical College Mitford Hospital on the occasion of World Hepatitis Day 2015. Doctors of all designation and nurses were included in the program. 175 doctors and 100 nurses participated in the program. The questionnaire of the program included a question of needle stick injury, its situation and

number. All the participants responded voluntarily. A total of 17 departments participated in the program.

Results: 51.5 % of the doctors and 80 % of the nurses reported a history of needle stick injury. They had single to multiple times injuries. Most of the injuries occurred in internee doctors and junior nurses. Recapping (61 %) with two hands is the main situation responsible for the injury in nurses while injury during suturing at surgery (50 %) was main cause among doctors.

Conclusion: NSI is a underreported problem in Bangladesh. Proper training in holding needle and medical waste disposal in need in addressing this problem.

P-1040

Taking charge of my life: advance care planning in patient with end stage liver disease

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Background: Advance care planning (ACP) is the process of planning for future health and personal care. It includes discussing patients' personal beliefs and goals for care with their loved ones and healthcare providers. This discussion is particular challenging in patient with end stage liver disease (ESLD) where the trajectory of the disease more uncertain than other terminal diseases. We aim to explore the experiences of advance care planning (ACP) among patients with ESLD and advanced hepatocellular carcinoma (HCC). **Method:** Patient with ESLD or advanced HCC with prognosis <1 year and their families were interviewed by ACP team using standardized questionnaires. We follow up these patients in clinics or via phone contact.

Result: Thirty-Four patients were engaged over 1 year. 15 patients (44 %) accepted ACP. Baseline characteristic between those who accepted/declined ACP discussions were similar. However patients who are sicker appeared to be more acceptance to ACP (Child Pugh Score 10.3 Vs 9.0, P = 0.07, median survival (days) 62.47 ± 67.31 Vs 114.76 ± 90.46, p = 0.076). All patients and families feels the ACP discussion was helpful in preparing them for future events. Major barriers of ACP discussion are lack of insight towards medical condition, denial from family members and caregivers refused to reveal diagnosis/prognosis to patient.

Conclusion: ACP discussion enhances positive emotions and facilitates communication between physician, patients and their family member to achieve a better end of life care. Improving patients and family knowledge, providing social and emotional support is the key factor to overcome barriers in ACP discussion.

P-1041

Advice of doctors and surrounding people influence decisions on hepatitis screening and treatment

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Introduction: It is important that we be able to identify HBV or HCV patients to prevent hepatocellular carcinoma. For comprehensive antiviral treatments, the following three steps should be followed: hepatitis screening, medical examination, and treatment. These steps are promoted using media such as informative advertisements.

Methods: We studied patients who followed the required steps and determined the most effective information source (social factor) for raising awareness of comprehensive treatments. We calculated the number of times patients accessed each of 18 information sources at each step (Examination 1), and ratios of source of information, which became the direct opportunity among sources of information where target persons contacted (Examination 2).

Results: We studied 182 patients undergoing viral hepatitis treatment from 11 medical institutions. More than 60 % of patients received “Recommendations from a family doctor”. “Recommendations from a public health nurse (PHN)”, “Recommendations from friends or family”, and “Recommendations from work colleagues” were the next most common (3.3–19.8 %). “Recommendations from a family doctor” had the greatest influence (76.9, 73.0, and 77.5 % at steps 1, 2, and 3, respectively). “Recommendations from a PHN” was also a strong factor (58.3, 26.3, and 64.3 %). Friends, family, and work colleagues were strong factors, and were more influential than a PHN at step 2. Media also raised awareness, but did not directly influence anyone (0–25.0 %).

Conclusion: Recommendations from a family doctor, friends, family, and work colleagues influence decisions regarding hepatitis screening, examination, and treatment.

P-1042

FSS and SF-36 in Asian patients with HCV G1 B receiving DCV + ASV and IFN + RBV intolerant/ineligible

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Aims: To assess the impact of 24 weeks of daclatasvir + asunaprevir therapy on overall quality of life (QOL) as measured by FSS and SF-36 in IFN-(±RBV) ineligible/intolerant patients from mainland China, Korea and Taiwan. (N = 159)

Methods: The FSS, a 9 question survey, measures fatigue utilizing a 7 point Likert scale, higher scores indicate greater fatigue. The SF-36 assesses generic health status across nine domains and includes a physical component summary (PCS) and mental component summary (MCS); higher scores for SF-36 indicate improved QOL. Surveys were completed by subjects every 4 weeks on treatment and post treatment weeks 4, 12 and 24.

Results: Overall baseline mean score for the FSS was 3.86. At follow up Week 12 (F12), mean change from baseline was -0.32 (SE = 0.14). When summarized by SVR12 status, responders (N = 143) had a mean decrease from baseline (3.81) at F12 of -0.35; SE = 0.14. Non-responders (N = 13) had a higher FSS mean baseline score (4.35) and, at F12, a mean change from baseline of 0.02 (SE = 0.45). Overall mean score for SF-36 PCS was 50.19 at baseline. At F12 mean change from baseline was 1.25 (SE = 0.56). SVR12 responders and non-responders had a mean change from baseline at F12 of 1.27 (SE = 0.57) and 0.96 (SE = 2.36) respectively. MCS demonstrated similar patterns with mean at baseline:

49.31 and mean change from baseline at F12 1.71 (SE = 0.75). At F12, MCS mean value increased from baseline for responders 2.25 (SE = 0.76) but decreased for non-responders -4.21 (SE = 3.00).

Conclusions: FSS and SF-36 demonstrated that patients maintained consistent QOL after 24 weeks of DCV + ASV treatment.

P-1043

How aware are chronic hepatitis B and C patients of their diseases?

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Introduction: This study evaluates the level of knowledge of chronic hepatitis patients about their disease in comparison to the general population. Method: 200 patients followed-up for chronic hepatitis and as a control group, 100 individuals who attended to the family medicine outpatient clinic for routine health control were randomly involved in the study. All participants were asked to fill the questionnaire we had prepared.

Results: Mean age and gender distribution of the chronic hepatitis and control groups were similar. The proportion of high school or university graduates was higher in the control group compared to the chronic hepatitis group (p = 0.03). The number of the participants who were aware of that hepatitis infection is transmitted via blood and sexual intercourse (p = 0.01) and the possible outcomes chronic hepatitis may cause (p = 0.03) were higher in the chronic hepatitis group compared to the control group. Both groups were categorized according to their educational levels. The rate of participants who were aware of the transmission routes and the possible outcomes of chronic hepatitis were significantly higher in the high school or university graduates. Chronic hepatitis group was also evaluated according to patients' follow-up durations. It was detected that the level of knowledge is not associated with the duration of follow-up.

Conclusion: Level of education is a strong effector for the awareness of transmission routes and possible outcomes of chronic hepatitis in both chronic hepatitis and control groups. The follow-up duration of chronic hepatitis patients does not affect their knowledge about their disease.

P-1044

The advanced liver disease pilot education program for nurses

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Background: In order to help reduce the costs of liver disease in Australia a 2013 expert report recommended the development of a nurse-led community based model of care run from a hospital based liver centre. Nurses need to be suitably skilled to take up this role. There is limited international and Australian experience in developing hepatology focused education programs which involve assessment of nurse skills and practice. The purpose of this study was to determine whether a mentorship based program is beneficial and feasible.

Methods: Seven hepatologist mentors (five from one of two tertiary teaching hospitals in Sydney and 1 from a regional setting) and seven nurses (five rural/regional and two Sydney-based) engaged in an 9 months program that included 6 modules comprising face-to-face teaching, skills workshop, clinical placement, on-line learning, mentoring and assessment. Hepatology nurses and hepatologists developed the advanced liver disease curriculum in collaboration with nurse academics from the University of Sydney. A mixed-method evaluation was developed including paper-based surveys and in-depth qualitative interviews with nurses and the hepatologist mentors.

Results: The face-to-face teaching, skills workshop, clinical placement and on-line learning were rated as highly beneficial and relevant to practice. Under mentorship nurses have begun to develop policies to improve care in their local services. The course has received credit recognition for a Primary Healthcare subject from the University of Sydney.

Conclusion: This education program has facilitated an extended scope of practice for nurses to improve access to care for people with advanced liver disease.

P-1045

Health perceptions amongst patients with fatty liver: a weighty issue

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Background/aim: The incidence of fatty liver and the metabolic syndrome are on the rise, and weight reduction is the first-line evidence-based intervention. Understanding knowledge gaps and beliefs of amongst patients with fatty liver is key to removing barriers and facilitating lifestyle modification to address the disease at the source.

Methods: 76 patients in a tertiary hospital Gastroenterology practice in Singapore, a multi-ethnic Asian population, were invited to participate in a self-administered questionnaire, comprising demographic data as well as knowledge, understanding and beliefs around weight reduction.

Results: The following groups were more likely to perceive a higher BMI as acceptable: Age more than 50 years: odds ratio (OR) 2.0 (95 % confidence interval (CI) 1.41–2.83); Presence of fatty liver OR 4.80 (95 % CI 1.69–13.68); Obesity; OR 4.27 (95 % CI 1.14–15.93); lower education states; OR 4.27 (95 % CI 1.14–15.93). Most patients (92.1 %) had correctly acknowledged the importance of weight loss and the means to do so, but admitted to difficulty implementing these strategies for various reasons including eating out most of the time (48.7 %) as well as being too busy to exercise (65.8 %).

Conclusion: It is of concern that patients at higher risk of metabolic complications; those with fatty liver, age more than 50 years, and obese, perceive a higher BMI as acceptable, hence putting them at greater risk.

Late Breaking Oral Presentation

LBO-01

Treatment of patients over age 80 by Sofosbuvir

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Aim: Experiences of patients treated by Sofosbuvir over age 80 is still limited. The safety and efficacy of the treatment for these population was elucidated.

Methods: Among 341 consecutively treated by Sovaldi or Harvoni, 32 were over age 80. Viral response and safety was studied.

Results: Of the 341, including 32 over age 80, none stopped Sovaldi (n = 111) or Harvoni (n = 230). During and after the follow up period, all but one who is 82 years old, genotype 2A, relapsed.

Conclusion: There are a few studies over age 65. But there is little information treated by Sofosbuvir over age 80. This result clearly indicated Sofosbuvir could be safely administered to over age 80, and nearly 100 % SVR could be expected in this population.

LBO-02

No influence on HbA1C level by acute suppression by Sofosbuvir

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Aim: Previous in vivo and vitro studies suggested HCV itself may worsen Diabetes. Thus, it was expected the parameter related DM, HbA1C level, may improve.

Results: Of 300 patients treated by Sofosbuvir, HbA1C level were serially tested before, during and after initiation of Sofosbuvir. Suppression of HCV replication was so obvious, e.g., HCV RNA became undetectable in all within 4–6 weeks. However, HbA1C level did not show any change in patients treated by Sofosbuvir.

Conclusion: Unlike interferon, nucleoside analogue, Sofosbuvir, has direct effect on virus replication. Thus, it was expected that viral suppression by Sofosbuvir may improve HbA1C level. However, hypothesis that HCV itself may worsen DM could not be substantiated by this study.

LBO-03

Long term effect of HCV eradication on serum lipids

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Aim: Previous in vivo and vitro studies suggested HCV itself may induce fatty change in the liver and hence worsen lipid metabolism.

Results: Of 50 patients who attained SVR by Sofosbuvir (Omata et al. J Viral Hep 2014, Mizokami et al. Lancet Inf Dis 2015), Total- and LDL-cholesterol were serially measured from 1 year before, during and to 2 years after Sofosbuvir treatment. Median pre-treatment level of Total- and LDL-cholesterol was 180 and 110 mg/dl, respectively. They increased to 220 and 148.

Conclusion: If the previous experimental studies on HCV and lipid metabolism suggested the improvement of lipid metabolism by eradication of HCV. On the contrary, after 2 years of virus replication, lipid metabolism seems worsened, judged by serum cholesterol levels. To see if NASH and NAFLD ensue, careful follow-up is needed for patients with the virus eradication.

LBO-04

Efficacy and safety of Sofosbuvir in HCV-related advanced liver cirrhosis. Data from RESiP Study

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Introduction: Patients with HCV-related advanced liver cirrhosis are almost impossible to be treated with interferon-based treatment regimens. Interferon-free, sofosbuvir-based treatment regimen seems to be the only hope of HCV eradication in such cases. We share our data of such cases from RESiP study.

Results: From Sep-2014 to Dec-2015, we have treated 82 patients with HCV-related advanced (CTP-B and C) with sofosbuvir and ribavirin for 24 weeks. About 57 % of patients were men and the mean age was 50 years. More than half (55 %) were treatment-experienced and the mean baseline HCV viral load was 1,679,812 IU/mL. Mean liver stiffness (on transient elastography by fibroscan) was 42.51 kPa. Mean ALT level was 90 IU/mL. Mean CTP score was 11. Mean MELD score was 13. During antiviral treatment, SVR12 was achieved in 66 % patients. The most frequent adverse events were fatigue, headache, insomnia, and nausea. Serious adverse events were uncommon: Jaundice, (1 %), GI upsets (2 %), worsening of ascites (4 %), hepatic encephalopathy (8 %), and marked anemia (11 %). Treatment discontinuations due to adverse events were in 9 % patients. Four (4.85 %) patients died; three developed new HCC and one died because of advanced encephalopathy. RESiP is an ongoing study and updated results will be presented in the meeting.

Conclusion: Sofosbuvir and ribavirin is an effective and reasonably safe in patients with HCV-related advanced liver cirrhosis. These patients need close monitoring during and after antiviral therapy for the tolerability and efficacy of therapy.

LBO-05

Influence of Sofosbuvir on eGFR (efficient glomerular filtration rate) less than 50 ml/min

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Aim: Renal impairment is one of obstacles to be treated by Sofosbuvir. Little information is available on safety and efficacy of patients

with eGFR less than 50 ml/min because none of 494 patients treated in clinical trials (Omata et al. J Viral Hep 2014, Mizokami et al. Lancet Inf Dis 2015) included that level of renal impairments.

Results: Of the 341 patients, 24 were below 50 ml/min at the beginning of the treatment, but above 30. During the treatment of 12 weeks, only minor fluctuation of eGFR in the range of 50–30 was observed. None of 24 stopped Sofosbuvir either by renal impairment or any other side effects.

Conclusion: Patients with eGFR less than 50 ml/min was excluded from the two registration trials in Japan. Sofosbuvir could be safely administered to the patients with eGFR below 50 ml/min but above 30 ml/min.

LBO-06

Surprisingly immediate and notable reduction of serum AFP, but not DCP by Sofosbuvir treatment

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Aim: As soon as viral suppression occurs by antiviral treatment, serum transaminases start to decline rapidly. We evaluated any other direct or indirect effect that could be observed in patients treated by Sofosbuvir.

Results: Of 300 patients treated by Sofosbuvir, biochemical parameters including liver related enzymes and tumor markers (AFP and DCP -des-γ-carboxy abnormal prothrombin) were serially tested before, during and after initiation of Sofosbuvir. DCP level only fluctuates and maintain its serum level, whereas AFP level dropped to a few nanogram range in all except one which exceeded 10 ng despite viral suppression. Interestingly, this patient was later turned out to have small HCC.

Conclusion: This study suggest two types of AFP production; inflammation (virus replication)-induced and tumor-induced. A lack of marked reduction to nanogram range by viral suppression (Sofosbuvir) may indicate high grade potential of HCC development.

LBO-07

A model for interaction between HBV and host cells derived from human induced pluripotent stem cells

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Background/aim: The objective is to determine whether human induced pluripotent stem cell-derived hepatic progenitor-like cells (iPS-HPC) and hepatocyte-like cells (iPS-Hep) can be used as a model for productive infection of HBV.

Methods: Human iPS cell lines were differentiated into hepatic lineage using 4-step protocol (Si-Tayeb 2010). FACS-sorted CD13+ CD133+ cells of the above-mentioned 3rd step (Yanagida 2013) were cloned, which were used as iPS-HPC host cells. Cells in cultured by

4-step protocol were used as iPS-Hep host cells. HBV for infection was obtained from the culture supernatant of HepG2.2.15 or HepG2 transfected with plasmids of HBV carrying a reporter gene (HBV-Rep).

Results: The expression of NTCP in iPS-HPC was 100-fold higher than that of hepatoma cell lines. Immunostaining showed that iPS-HPC produced α -fetoprotein, HNF4a, and NTCP. HBV-cccDNA and pregenomic RNA were detected in iPS-HPC. HBeAg and HBsAg were detected in the culture supernatant at day 13 after infection. The production of reporter gene in iPS-HPC infected with HBV-Rep was significantly higher than that of hepatoma cell lines. The induction of interferon-stimulated genes in iPS-HPC treated with IFN α was significantly higher than that of HepG2-NTCP. The expression of NTCP in differentiated iPS-Hep was significantly increased as compared with iPS-HPC. The expressions of cccDNA, pregenomic RNA, HBeAg, and HBsAg in iPS-Hep were significantly higher than those of iPS-HPC. The infection of HBV with iPS-Hep was significantly suppressed by several anti-viral drugs.

Conclusions: Human iPS-derived cells could be a promising model for the research on life cycle of HBV and antiviral strategies.

LBO-08

Natural history of children with chronic hepatitis B: the observational multi-center study in Japan

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Background and aims: To plan the long-term management of childhood HBV infection, it is necessary to clarify the natural history of children with HBV infection in each country.

Methods: A retrospective multi-center study of children with chronic HBV infection who were diagnosed at age below 15 years was carried out in 18 hospitals.

Results: We reviewed 548 Japanese children with HBV infection including 381 of mother to children transmission (MTCT), 154 of horizontal transmission and 13 of blood transfusion. Children with horizontal transmission were more frequently infected with non-C genotypes such as genotype A and B, and received interferon therapy more frequently than those with MTCT. The average rates of HBeAg seroconversion at 15 years of age were 39 % in children with horizontal transmission and 43 % in those with MTCT. In children with horizontal transmission, the proportion of those with hepatitis below 15 years of age was higher than in children with MTCT. Among patients presenting with chronic hepatitis HBeAg seroconversion occurred within 3 years after the onset of hepatitis in 30 % of patients with horizontal transmission and in 40 % of those with MTCT. The occurrence of HCC was noted in 14 patients below 30 years of age.

Conclusions: Hepatitis occurred at all age of patients with childhood HBV infection, and in approximately 40 % of them, HBeAg seroconversion occurred before the age of 15 years. Patients with childhood HBV infection may carry a risk to develop HCC from childhood to adulthood and their lifelong follow-up may be mandatory.

LBO-09

HBsAg variability on response to Pegylated-interferon plus Tenofovir combination therapy

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New combination therapy of pegylated interferon (PEG-IFN) with tenofovir (TDF) is assessed in order to improve the rate of HBsAg loss. In this study we investigate the S-gene variability of chronic hepatitis B (CHB) patients treated with PEG-IFN plus TDF in order to determine the role of HBsAg variants on response to treatment. Patients received 180 μ g of PEG-IFN/week plus 300 mg of TDF/day during 48 weeks. Sustained virologic response (SVR) was defined as HBV DNA <2000 IU/mL 48 weeks after end of therapy (EOT). HBsAg-encoding gene from 40 patient's serums at baseline was PCR-amplified, cloned and sequenced. The median age of patients included was 44 years, 32 patients (80 %) were male and 12 (30 %) were HBeAg-positive. 48 weeks after end of treatment (EOT), 9/40 patients (22 %) achieved a SVR and 5 of them have a HBsAg loss. Comparison of S-gene variability assessed by direct sequencing indicated a higher but non-significant percentage of mutated residues in N-SVR vs SVR ($p = 0.07$). Clonal analysis of N-SVR vs SVR patients revealed a higher residue substitutions frequency in the major hydrophilic region (MHR) ($p = 0.002$), especially in the "a" determinant region ($p = 0.007$). Overall, in patients treated with PEG-IFN + TDF, 22 % achieved a SVR and 12 % achieved HBsAg loss. N-SVR patients showed more variability along the S protein. The accumulation of residue substitutions in and around the "a" determinant at baseline should be a sensitive predictor of non-response to combination of PEG-IFN and TDF therapy in CHB patients.

LBO-10

5-year telbivudine optimized treatment for CHB shows sustained viral suppression and low resistance

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Background: Two-year telbivudine (LDT) optimization strategy based on “ROADMAP CONCEPT” has been demonstrated to be superior to LDT monotherapy in terms of viral suppression and resistance for HBeAg positive, nucleos(t)ide-naïve CHB patients. Here, we present the results after up to 5 years (260 weeks) of continued optimized therapy vs. monotherapy.

Methods: Six hundred and six adult patients with HBV DNA 10^5 copies/ml, ALT 2–10 × ULN and compensated adult HBeAg-positive CHB were randomized to the Optimize (n = 302) or Mono (n = 304) group. From week 104, therapy was stopped for patients with HBeAg seroconversion and HBV DNA <300 copies/mL for at least 48 weeks (Figure).

Results: Sixty-eight percent of patients in the Optimize group had ADV added due to suboptimal response. Among patients continuing in the third year (Optimize, n = 276; Mono, n = 266), 90.9 % of Optimize-treated vs. 75.9 % of Mono-treated patients achieved HBV DNA <300 copies/ml (P < 0.0001), and 3.3 % of Optimize-treated vs. 42.5 % of Mono-treated patients occurred resistance to LDT (P < 0.0001) after up to 260 weeks of treatment. The rates of HBeAg seroconversion, HBeAg loss and ALT normalization were comparable between the two groups (29.7 vs. 33.1 %; 41.3 vs. 45.1 %; 85.3 vs. 84.6 %). HBsAg loss occurred in 5 patients (1.8 %) in Optimize group and 4 patients (1.5 %) in Mono group, respectively. Both treatment regimens were well tolerated.

Conclusions: LDT optimization strategy based on “ROADMAP CONCEPT” provides sustained viral suppression with low resistance during long-term treatment of HBeAg-positive CHB patients.

LBO-11

A randomized controlled trial of glycyrrhizin in chronic hepatitis B with severe acute exacerbation

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Background/aim: Severe acute exacerbation of chronic hepatitis B (CHB-AE) leads to a significant mortality rate despite the prompt treatment with nucleos(t)ide analogues. Glycyrrhizin can lower serum levels of transaminases and improve liver histology in chronic active hepatitis, but limited data are available in acute hepatitis. This study aimed to evaluate the efficacy and safety of glycyrrhizin in the treatment of CHB-AE.

Methods: Sixty patients with CHB-AE were randomly treated with tenofovir plus intravenous glycyrrhizin (group A, n = 30) or with tenofovir alone (group B, n = 30). Primary endpoints were the improvement of serum levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) and model for end-stage liver disease

(MELD) score. Secondary endpoint was overall mortality or receiving liver transplantation by week 24.

Results: Patients in group A had significant reductions of serum AST and ALT levels from baseline at day 3, 5, 8 and 15 than those in group B (all p < 0.05). A higher proportion of improvement in MELD score was found in group A than in group B at day 8 (83 vs. 52 %, p = 0.021). By week 24, one (3.3 %) of group A patients and 4 (13.3 %) of group B patients died (n = 4) or received liver transplantation (n = 1) (p = 0.177). Multivariate analysis identified baseline MELD score (p = 0.021) as an independent factor for mortality or liver transplantation. There were no differences in the rates of grade 3 hypertension, hypokalemia and ascites between two groups.

Conclusion: Early combined glycyrrhizin with tenofovir can be used safely and be helpful for patients with CHB-AE.

LBO-12

A novel therapy for chronic hepatitis B; proof of concept, mechanism, clinical trial and registration

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Impaired and distorted immunities of chronic hepatitis B (CHB) patients are considered to be related with ongoing HBV replication and progressive liver damages, however, commercially-available antiviral drugs are endowed with limited efficacy, lack of sustained effects, considerable adverse reactions and need of prolonged usage. Even, upregulation of host immunity of CHB patients by different immune therapeutic agents have not also shown viable therapeutic promises in CHB patients during last 3 decades. To retrieve evidences of proof of concept, we used a combination of HBsAg/HBcAg-based therapeutic vaccine in HBV transgenic (TM) and patients with CHB. A series of experiments were accomplished to elucidate the nature, type, magnitude, and durability of therapeutic vaccine-induced immunities in chronic HBV-infected subjects. A temporal relation between therapeutic effects of therapeutic vaccine versus kinetics of HBsAg and HBcAg-specific immunities were also evaluated from cytokine production assays, T cell proliferation experiments, and cytotoxic T lymphocytes (CTL) evaluation. The safety, antiviral capacity and liver protection potential of this therapeutic vaccine were confirmed in phase III clinical trial by comparing those with another group of patients receiving pegylated interferon. Sustained persistence of HBcAg-specific immunity was seen in responders to vaccine therapy. These fundamental and clinical studies led to the registration of this therapeutic agent before 2 weeks. Note: The 1st and 2nd authors have equally contributed to this study.

LBO-13

Comprehensive analyses of mutations and hepatitis B virus integration in hepatocellular carcinoma

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Background and aims: Genetic alterations in specific genes are critical events in carcinogenesis and hepatocellular carcinoma (HCC) progression. However, the genetic alterations responsible for HCC development, progression, and survival are unclear.

Methods: We investigated the essential difference in genetic alterations between HCC and adjacent non-HCC tissues using next-generation sequencing technology.

Results: We found recurrent mutations in several genes such as TERT (65 % of the total 104 HCCs), TP53 (38 %), CTNNB1 (30 %). TERT promoter mutations were associated with presence of hepatitis C virus (HCV) infection ($P = 0.003$), and absence of hepatitis B virus (HBV) infection ($P < 0.0001$). In hepatitis B surface antigen (HBs Ag) positive HCC without TERT promoter mutations, HBV integration into TERT locus was found in 47 % patients and was mutually exclusive to TERT promoter mutations. Most (89 %) HBV integrants were in the HBx region. In patients with prior HBV infection, breakpoints were randomly distributed through the HBV genome, and only 12.5 % were found in the HBx gene. TP53 mutations were associated with HBV infection ($P = 0.0001$). CTNNB1 mutations were associated with absence of HBV infection ($P = 0.010$). Moreover, TERT promoter mutation was significantly associated with shorter disease-free survival ($P = 0.005$) and poor overall survival ($P = 0.024$).

Conclusions: Gene alterations in TERT promoter, TP53, CTNNB1, and HBV integration were closely associated with HCC development, and mutations in TERT promoter are related to poor prognosis. These results are useful for understanding the underlying mechanism of hepatocarcinogenesis, diagnosis, and predicting outcomes of patients with HCC.

LBO-14

Lower survival with cirrhosis in hepatitis B virus (HBV)-related hepatocellular carcinoma (HCC)

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Background: HBV is the leading cause of HCC worldwide and can cause HCC in the absence of cirrhosis. Few studies have compared cirrhotic and non-cirrhotic HBV patients with HCC. We report the largest cohort study of HBV-related HCC focusing on cirrhosis status.

Methods: This is a retrospective cohort study of 3821 patients with HBV-related HCC diagnosed 2000–2015 at four medical centers: 485 from a US center, 500 from a Korea center, and 2952 from two Taiwan centers. Cirrhosis diagnosis was based on biopsy or clinical,

laboratory, or imaging evidence of impaired synthetic function or portal hypertension.

Results: Almost all (99 %) patients were Asian, and the majority were male (73 %) and cirrhotic (70 %). Of the cirrhotic patients, 67 % were Child-Pugh (CP) class A. Tumor characteristics and treatments are shown in Table 1. Patients with cirrhosis had larger and more often multifocal tumors, and were less likely to receive treatment. Four-year survival was lower in patients with cirrhosis (Fig. 1), in all sites. Four-year survival was lower among cirrhotic patients in Taiwan than the other sites but was identical in all sites among non-cirrhotic patients. On multivariate Cox regression including age, sex, treatment type, symptomatic presentation, BCLC stage, Child-Pugh score, site, and date of diagnosis, cirrhosis was associated with decreased survival (HR 1.25; 95 % CI 1.09–1.44; $p = 0.002$).

Conclusions: Cirrhosis was associated with lower treatment eligibility and overall survival in this international cohort of HBV-related HCC cases, though four-survival rates remained high at approximately 40 % and 60 % for cirrhotic and noncirrhotic groups, respectively.

LBO-15

Survival unchanged in hepatitis B virus (HBV)-related hepatocellular carcinoma (HCC) from 2000 to 2015

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Background: HBV is the leading cause of HCC worldwide. It is unclear to what extent recent advances in HBV and HCC management have influenced patient outcomes. We report the largest cohort study of HBV-related HCC to investigate temporal trends.

Methods: This is a retrospective cohort study of 3821 patients with HBV-related HCC diagnosed 2000–2015 at four medical centers: 485 from a US center, 500 from a Korea center, and 2952 from two Taiwan centers.

Results: Almost all (99 %) patients were Asian, and the majority were male (73 %) and cirrhotic (70 %). We analyzed two time periods: 2000–2009 ($n = 2129$) and 2010–2015 ($n = 1692$). Patient and tumor characteristics and treatments are shown in Table 1. More recently-diagnosed patients were older but less often had cirrhosis. Tumor size and BCLC stage increased over time. Taiwan patients were older and had larger tumors than those in the other sites. Four-year survival (chosen to ensure follow-up data were available) is shown in Fig. 1. Overall and in each individual site, survival did not differ based on diagnosis date. When comparing survival based on site, survival was similar in the 2000–2009 period ($p = 0.67$) but in the 2010–2015 period there was a trend toward lower survival in the Taiwan sites ($p = 0.0503$).

Conclusions: Recently-diagnosed patients had similar four-year survival to those diagnosed earlier. Further research will identify reasons for the lack in improvement in survival over time.

LBO-16

Cancer vaccine development for hepatocellular carcinoma: HEPAVAC

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The HEPAVAC Consortium aims at developing a new immunotherapy for hepatocellular carcinoma (HCC). The consortium includes nine European Partners from academia and biotech industry with complementary and substantial expertise. The project has started in September 2013 and is supported by the European Commission's 7th Framework Program (<http://www.hepavac.eu>). HCC/normal adjacent tissue matched samples have been collected for HLA immunopeptidome analysis. 17 HCC samples from HLA-A*02⁺ patients and 15 samples from HLA-A*24⁺ patients have been analyzed by mass spectrometry (LC-MS/MS). RNA-expression profiles have been established for 12 HCC samples and compared to >140 normal tissue samples from healthy organs. A total of 9051 tumor-associated peptides (TUMAPs) have been identified from HLA-A*02⁺ samples, while a total of 3286 different HLA-A*24-restricted TUMAPs have been identified from HLA-A*24⁺ samples. Of these, 33 HLA-A*02⁺ TUMAPs and 33 HLA-A*24⁺ TUMAPs have been selected, the corresponding peptides synthesized and undergone preclinical assessments. The most promising TUMAP candidates show selective expression in HCC as compared to normal tissues. In addition, about 1500 HLA-DR TUMAPs have been identified in HCC samples. A total of 16 newly identified HCC-specific epitopes (7 HLA-class I A*02; 5 HLA-A*24 and 4 HLA class II) have been selected for the vaccine cocktail and are currently synthesized according to GMP standard. In parallel, preclinical studies assessing the formulation and combination of the immunological RNA-based adjuvant (RNAdjuvant[®]) with peptide cocktails are underway. A multi Center phase I/II clinical trial in early HCC patients is expected to start in July 2016.

LBO-17

Comparison between liver transplantation and sorafenib for BCLC B or C hepatocellular carcinoma

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Background and aims: For unresectable hepatocellular carcinoma (HCC) patient, sorafenib is the only systemic treatment showing a survival gain, which, however, is only several months. We have recently reported that beyond—Milan criteria (MC) patients with low MoRAL score [=11 × root(protein induced by vitamin K absence-II) + 2 × root(alpha-fetoprotein)] experienced 5-year survival rate exceeding 80 %. Herein, we investigated whether LDLT offers better treatment outcome than sorafenib for HCC patients with BCLC B or C stage.

Methods: A total of 325 consecutive patients (122 patients in LDLT group; 203 patients in sorafenib group) with BCLC B or C stage who were treated with either LDLT or sorafenib between 2005 and 2014 at a single tertiary hospital were included. Patients with extrahepatic metastasis were excluded.

Results: When the baseline characteristics were balanced using IPW, the sorafenib group experienced significantly higher risk of tumor progression (hazard ratio [HR], 6.4; P < 0.0001) and death (HR 9.2; P < 0.0001) compared to the LDLT group. Median OS was 34.8 months in the LDLT group and 8.2 months in the sorafenib group (P < 0.0001). Increase in OS from LDLT over sorafenib was more predominant among those patients with low MoRAL score (<314.8) (HR, 13.6; P < 0.0001), compared to those with high MoRAL score (>314.8) (HR, 4.0; P = 0.002).

Conclusions: For HCC patients with BCLC B or C, LDLT exhibited significantly longer TTP and OS compared to sorafenib. BCLC B or C patient with a low MoRAL score might be a good candidate for LDLT rather than sorafenib, if there is a willing living-related donor.

LBO-18

Decreased miR-425 induced inflammatory cytokine production in primary biliary cholangitis

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Background: Primary biliary cholangitis (PBC), formally known as primary biliary cirrhosis, is an autoimmune liver disease of unknown pathogenesis. Moreover, therapeutic targets for the autoimmunity of PBC are not yet found. Since CD4⁺ T cells are known to play a pivotal role in the immunological disorder of PBC, we analyzed microRNA (miRNA) and mRNA of CD4⁺ T cells integrately to reveal its pathogenesis.

Methods: Clinically and pathologically diagnosed seven PBC patients and seven healthy controls, who agreed to provide samples with written informed consent, were enrolled in this study. Then, we analyzed the expression profile of miRNA and mRNA in CD4⁺ T cells of PBC patients and controls by microarray and qRT-PCR using a gene set enrichment analysis (GSEA). The biological function of differentially expressed and GSEA-enriched miRNAs was evaluated by miRNA overexpression assay, reporter assay, and CD3 stimulation assay using cultured cells.

Results: An integral miRNA-mRNA analysis revealed 4 decreased miRNAs (miR-181a, -181b, -374b, -425) coordinately dysregulated T cell receptor (TCR) signaling pathway in CD4⁺ T cells of PBC.

Especially, N-Ras in the upper stream of TCR signaling pathway was targeted by 4 decreased miRNAs. In vitro assays revealed miR-425 downregulated N-Ras expression and therefore suppressed production of inflammatory cytokines (IL-2, IL-10, and IFN- γ).

Conclusion: Decreased miR-425 in CD4+ T cells upregulates N-Ras and induces the inflammation through dysregulation of TCR signaling pathway in PBC. The restoration of decreased miR-425 and/or downregulation of N-Ras expression in CD4+ T cells could be novel therapeutic options for PBC.

LBO-19

Granulocyte colony stimulating factor improves survival of patients with decompensated cirrhosis

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Granulocyte colony stimulating factor (GCSF) therapy can mobilize bone marrow stem cells for tissue regeneration and has been shown to benefit patients with liver disease. We evaluated the efficacy of GCSF therapy in decompensated cirrhosis in an open labelled randomized control trial. Consecutive patients with decompensated cirrhosis of mixed etiologies were randomized to receive 5-day course of GCSF (5 $\mu\text{g}/\text{kg}/\text{day}$) plus standard medical therapy for 6 months (Group-A) or standard medical therapy alone for 6 months (Group-B). At the end of 6 months their survival were compared. A total of 240 patients were included (median age 53, range 31–76, 84 % males). Their baseline median Child-Turcotte-Pugh (CTP) score and MELD score were 10 (range 7–13) and 17 (range 6–33) respectively. They were randomized to GCSF plus standard medical therapy (Group-A, n = 120) and standard medical therapy alone (Group-B, n = 120). Baseline characteristics were similar in both the groups. No significant adverse effect requiring stoppage of therapy or dose reduction was seen. At the end of 6 months of follow up in group-A, 16 patients had died and 10 were lost to follow up, thus 94/120 (78 %) patients were able to complete the follow up. In group-B, 17 patients had died and 28 were lost to follow up, thus 75/120 (62 %) patients were able to complete the follow up. Thus according to intention-to-treat analysis, survival was significantly better in group-A as compared to group-B ($p = 0.007$ by Log rank test). GCSF therapy improves survival in patients with decompensated cirrhosis

LBO-20

A randomized, double-blind, placebo-controlled trial of silymarin for the treatment of NASH

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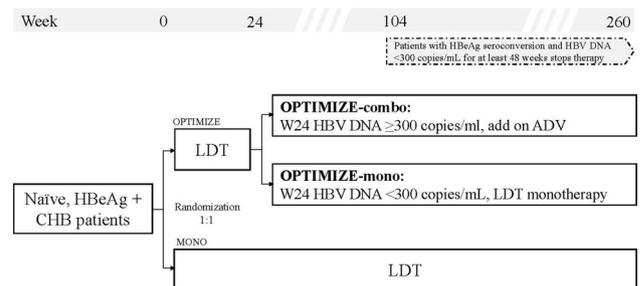
Background: Silymarin has been used as herbal remedy for liver diseases.

Methods: This is a randomized, double-blind, placebo-controlled study of silymarin 700 mg t.i.d. for the treatment of non-alcoholic steatohepatitis (NASH). All included patients had biopsy-proven NASH, were given lifestyle advice, and received either silymarin or

placebo for 48 weeks. A repeat liver biopsy was performed at the end of the study. Histology was reported using the NASH Clinical Research Network scoring system (Fig. 1).

Results: Of the 148 subjects who underwent screening, 99 subjects were randomized. Eighty-nine subjects (89.9 %) underwent end-of-treatment liver biopsy (44 in the silymarin group and 45 in the placebo group). There was no significant difference in the primary efficacy outcome (defined as ≥ 30 % improvement in NAFLD activity score) in the silymarin and placebo groups (36.4 vs. 28.9 %, $p = 0.452$). However, there was significant difference in the changes in fibrosis stage in the silymarin group compared with the placebo group (-0.205 vs. $+0.111$, $p = 0.026$), and more subjects receiving silymarin experienced fibrosis improvement compared with placebo (25.0 vs. 6.7 %, $p = 0.021$). The number needed to treat for fibrosis improvement was 5. Additionally, more subjects in the silymarin group had ≥ 30 % improvement in liver stiffness measurement compared with the placebo group (26.3 vs. 2.6 %, $p = 0.003$). There was no significant difference in adverse events in the silymarin and placebo groups, and none of the adverse events were deemed related to the study drug.

Conclusions: Silymarin treatment was associated with significantly greater fibrosis improvement compared with placebo in patients with NASH.



For LDT monotherapy, ADV will be added if viral breakthrough is confirmed.

Figure: The design of EFFORT study (NCT00962533, NCT01529255). LDT, telbivudine; ADV, adefovir.

LBO-21

The predictive value of HBV cccDNA for antiviral therapy in children with CHB

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Background: To assess the relationship between HBV cccDNA level in the liver tissues and the recurrence after the cessation of therapy in children with chronic hepatitis B(CHB).

Methods: 3 children with HBeAg-positive CHB were enrolled, who were treated with subcutaneous alfa-interferon at a dose of 1–5 MIU/m² 3 times weekly for 48 weeks, which were followed up for 24 weeks after the cessation of therapy. Serum HBV surface antigen and HBV DNA were measured at baseline, the week 48 of treatment and 24 weeks after the cessation of therapy. Biopsies from 3 patients were available for both intrahepatic total HBV DNA and cccDNA testing at week 48 of treatment time points.

Results: Among three patients, two patients' HBVcccDNA level of liver tissues were more than 0.05 copies/cell and 3 positive signals in situ detection of liver tissue at week 48, the serum HBV DNA level from 1.0×10^2 copies/mL and 0.9×10^2 copies/mL at week 48

rebound to 3.2×10^3 copies/mL and 4.6×10^4 copies/mL, respectively, during the 24 weeks follow-up, but one patient of them, whose HBVcccDNA level of liver tissues at week 48 less than the above value, did not rebound of HBV DNA level during the 24 weeks follow-up.

Conclusions: HBVcccDNA level of liver tissues more than 0.05 copies/cell and 3 positive signals in situ detection of liver tissue at week 48, might be a predict data of relapse after the cessation of therapy, but the application in clinical practice should be further studied.

LBO-22

International Radiofrequency Ablation (RFA) Training Program

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RFA is now performed in many facilities throughout the world. However, its skills and outcomes are different from operator to operator, or from institution to institution. Our institution is the highest volume center of RFA in Japan now. To disseminate skills and know-hows for RFA, we held domestic RFA training programs six times and 99 doctors participated in. This time we expanded the scope to foreign doctors. There were 13 participants from abroad. We had a 7-days-long program in which we set lectures, live demonstrations, and case studies as main three pillars as is done in the domestic programs. During the program, we performed RFA on 15 cases and ablated 28 tumors in total in five treatment days. These were a wide variety of cases: cases of newly diagnosed cancer not difficult to ablate judging from size and location, a tumor just below the diaphragm requiring artificial ascites, a tumor in the caudate lobe, a tumor adjacent to the heart, a tumor just next to portal vein or hepatic vein, a tumor over 5 cm, more than five tumors, hepatic metastasis, etc. From these cases, we demonstrated the importance to have appropriate patient posture, usefulness of our original dedicated probe for interventional procedures and our RFA dedicated operation table, and ways to perform ablation under Sonazoid guidance, and with multimodality fusion imaging. A questionnaire survey revealed overwhelmingly positive feedback from the participants. We are having the 2nd international training program in February 2016.

LBO-23

RFA for metastatic liver tumor due to colorectal cancer a retrospective matched control study

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Objective: The efficacy of radio frequency ablation therapy (RFA) for metastatic liver tumor due to colorectal cancer is still unknown.

Methods: In this retrospective study we enrolled 161 colorectal cancer patients with synchronous liver metastasis. Among these patients, we matched the patient background as follows: resected

primary sites, age, sex, tumor numbers, and tumor size. Finally, 20 patients were included in the RFA group, and 20 patients were included in the controls. We compared the overall survival (OS) between the two groups and analyzed the predictive factors which affected on OS.

Results: The median follow-up period was 2.7 years. The median age was 66.9 years and 23 patients were male (57.5 %). There were no significant differences about patients' backgrounds between the two groups, because the two groups were matched. The mean tumor numbers were 5 in the RFA group and 4.6 in the controls ($P = 0.71$). The mean tumor diameter were 39.9 mm in the RFA group and 44.8 mm in the controls ($P = 0.59$). During the observation period, 4 patients of the RFA group did not receive any chemotherapy. The cumulative survival rates significantly differed between the two groups ($P < 0.01$); 100, 100, and 77.0 % at 1, 2, and 3 years, respectively, in the RFA group and 80.0, 48.1, and 34.4 % in the controls. Multivariate analysis indicated only receiving RFA as independent factors (HR 0.14, 95 % CI 0.04–0.49), $P < 0.01$).

Conclusion: RFA seems to be a good treatment option for metastatic liver tumor due to colorectal cancer.

LBO-24

Activity of hepatic stellate cells in chronic hepatitis C virus infection

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Background: Alpha-smooth muscle actin (α -SMA)-positive hepatic stellate cells (HSC S) are pericytes responsible for fibrosis in chronic liver injury. The glial fibrillary acidic protein (GFAP), commonly expressed by astrocytes in the central nervous system, is expressed in vivo in the liver in a subpopulation of stellate cells.

Aim: To study the activity of HSC S in chronic hepatitis C (CHC).

Methods and results: With immunohistochemistry and a semi-quantitative scoring system, the expression of α -SMA and GFAP on HSC S in liver biopsies from patients with pure CHC ($n = 34$), hepatitis C virus-induced cirrhosis ($n = 24$), mixed CHC-schistosomiasis ($n = 11$) and normal controls ($n = 10$) were analyzed. The immuno-reactivity of α -SMA and GFAP in peri-sinusoidal, periportal and peri-central areas was assessed. α -SMA and GFAP-positive HSC S were significantly increased in all diseased groups compared with normal controls. In pure CHC with or without cirrhosis.

Conclusion: Early activation of HSC S in CHC patients seems to be an early indicator of hepatic fibrogenesis.

LBO-25

PREB and Surfeit 4 are involved in HCV replication compartment by interacting with NS4B

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It has been proposed that HCV NS4B protein triggers the membranous HCV replication compartment, but the underlying molecular mechanism is not fully understood. Here, we screened for NS4B-associated membrane proteins by tandem affinity purification and proteome analysis and identified 202 host proteins. Subsequent small interfering RNA screening in replicon cells identified prolactin regulatory element binding (PREB) and Surfeit 4, factors reported to regulate recruitment of COPII and COPI, as a novel HCV host cofactor for regulating HCV replication, respectively. The interaction between the cofactors and NS4B was confirmed by immunoprecipitation, immunofluorescence and proximity ligation assays. They colocalized with double-stranded RNA and the newly synthesized HCV RNA in HCV replicon cells. Furthermore, they shifted to detergent-resistant membranes (DRM), where HCV replication complexes reside, in the presence of NS4B expression in Huh7 cells. However, their mutant lacking the NS4B binding region could not colocalize with double-stranded RNA and did not shift to the DRM in the presence of NS4B. These results indicate that PREB and Surfeit 4 locate at the HCV replication complex by interacting with NS4B. Their silencing inhibited the formation of membranous HCV replication compartment and increased the protease and nuclease sensitivity of HCV replicase proteins and RNA in DRM, respectively. The present data show that the PREB and Surfeit 4 are recruited into the HCV RNA replication complex by interaction with NS4B, and that they promote HCV RNA replication by increasing the formation of membranous HCV replication complex, thus providing new insights into HCV host cofactors.

LBO-26

Regional differences in hepatitis C treatment in Japan: a study using a nationwide database

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Background: Over 300,000 individuals die from hepatitis C-related liver disease annually worldwide. Nationwide standardization of treatment is important to control hepatitis C epidemics and to reduce the occurrence of liver diseases with mortality risks. However, regional differences in hepatitis C treatment are still unclear.

Methods: We conducted a retrospective cohort study using the Japanese interferon database. Individuals with chronic hepatitis C (genotype 1/2) registered from December 2009 to April 2013 were identified from the database. The sustained virological response (SVR) rate was calculated for each genotype. Confounding variables were selected using stepwise multivariate logistic regression, and adjusted odds ratio was estimated for each prefecture.

Results: From 36 prefectures, 16,349 cases were registered in the database during the study period. According to the inclusion and exclusion criteria, 11,653 cases (genotype 1 7950 cases; genotype 2, 3703 cases) were included in the analysis. The SVR rates for each prefecture as follows: 48.4 [30.0–63.0 %] for genotype 1 and 80.4 [55.0–100.0] for genotype 2. The range of adjusted odds ratio for the

reference prefecture (prefecture no. 1) were 0.66–2.13 for genotype 1 and 0.36–2.63 for genotype 2.

Conclusions: The SVR rates for each prefecture varied and the range of adjusted odds ratios was relatively wide; there may be regional differences in hepatitis C treatment in Japan. Additional studies considering medical situations in each prefecture may be useful to improve hepatitis C treatment in Japan.

LBO-27

Residual RAVs after Daclatasvir and Asunaprevir combination therapy: Multicenter study in Japan

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Background and aims: Treatment failure after Daclatasvir (DCV)/Asunaprevir (ASV) combination can induce resistance-associated variants (RAVs) in NS3 and NS5A region of hepatitis C viruses. The aim of the present study was to characterize the RAVs after DCV/ASV failure.

Methods: Sixty-two genotype 1b patients who failed to achieve sustained virological response (SVR) by 24 weeks of DCV/ASV therapy at 93 hospitals in Japan. The RAVs were determined by direct sequencing, and the frequencies of RAVs were compared to 858 treatment naïve patients.

Results: The frequency of RAVs at position T54, A156, and D168 in NS3 region were significantly higher in patients after DCV/ASV failure compared to treatment naïve patients (7.5 vs 2.3 %, 1.8 vs 0.2 %, 68 vs 4.3 %, $p = 0.02, 0.04, <0.001$). Most common RAVs at D168 position was D168E variant (69 %). The frequency of RAVs at position L31 and Y93 in NS5A region were significantly higher in patients after DCV/ASV failure (77 vs 3.5 %, 70 % vs 19 %, both $p < 0.001$). All Y93 RAVs was Y93H variants. A significant proportion of patients after DCV/ASV failure had triple RAVs such as D168 and L31 and Y93 (54 vs 0.35 %, $p < 0.001$), and highly resistant triple RAVs in NS5A region such as L31 and Q54 and Y93 (32 vs 0.35 %, $p < 0.001$).

Conclusion: Treatment failure of DCV and ASV combination therapy could induce highly complex multi-drug resistant viruses. The presence of these RAVs should be taken into account in the selection of rescue therapy for patients DCV/ASV failure.

LBO-28

Viral kinetics in HCV genotype 3 patients treated with Sofosbuvir in Pakistan. Data from RESiP Study

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Introduction: Sofosbuvir has been found to be highly effective in the treatment of HCV. Here we report status of viral kinetics in HCV

genotype 3 patients treated with Sofosbuvir + Ribavirin with or without Interferon in Pakistan.

Results: PCR was considered to be negative if viral load was less than 15 IU/mL. On analysis of data of 725 patients treated in RESiP study, mean baseline HCV viral load was 1766544 IU/mL. During antiviral treatment, PCR became negative after 1 week in 85 %, after 2 weeks in 87 %, after 4 weeks in 96 %, after 12 weeks in 97 %, and after end of antiviral treatment in 98 % patients, and it remained negative in 84 % 12 weeks after the end of antiviral treatment. RESiP is an ongoing study and updated results will be presented in the meeting.

Conclusion: Sofosbuvir is highly effective treatment HCV genotype 3; most patients become negative within 1–2 weeks of treatment and remain negative during treatment. About 15–16 % patients relapse within 12 weeks of end of treatment.

LBO-29

RESiP study: real-life experience with Sofosbuvir in Pakistan

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Introduction: RESiP is an observational study describing real-life experience of treatment of HCV genotype three patients with Sofosbuvir in Pakistan.

Results: From Sep-2014 to Dec-2015, we have treated 725 patients with sofosbuvir and ribavirin with and without pegylated interferon for 12 or 24 weeks respectively, depending upon eligibility of interferon. Overall, 55 % of patients were men and the mean age was 46 years. More than half (56 %) had non-CC IL28B genotypes and the mean baseline HCV viral load was 17,66,544 IU/mL. Out of 725 patients, therapy has completed in 181 patients with 98 % ETR; 47 patients has completed 12 weeks post therapy follow-up and SVR12 was achieved in 85 % patients. SVR12 was 100 % in new patients without cirrhosis, 92 % in treatment-experienced patients without cirrhosis, 88 % in new patients with cirrhosis, and 79 % in treatment-experienced patients with cirrhosis. The most frequent adverse events were fatigue, headache, insomnia, and nausea. Serious adverse events were uncommon: 3 % with the dual regimen, and 4 % with triple regimen. Treatment discontinuations due to adverse events were rare and occurred with similar frequency in both treatment arms. Significant laboratory abnormalities were 10 % in dual therapy and 18 % in triple RESiP is an ongoing study and updated results will be presented in the meeting.

Conclusion: Sofosbuvir and ribavirin is highly effective and safe in patients with HCV genotype 3 in with or without interferon. Treatment experienced patients with cirrhosis respond relatively low.

LBO-30

Effect of entecavir therapy in 50 children with chronic hepatitis B in real life longitudinal study

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Background: Treatment of chronic hepatitis B (CHB) in children with nucleoside analogs is controversial due to unsatisfied results and the risk of resistance. We evaluated the long-term (up to 6 years) efficiency of entecavir (ETV) treatment in CHB patients infected in childhood.

Methods: This retrospective study collected data of 50 consecutive patients with CHB, who were infected with HBV in the first years of their life and who have begun treatment with ETV. All examined patients were treated with ETV monotherapy in the dose 0.5 or 1 mg/day. The effectiveness of the therapy was evaluated by indicating the concentration of HBV DNA and ALT activity and before, during and after the treatment. To investigate the dynamics of HBV DNA and ALT activity over time and its association with considered factors the Generalized Estimating Equation (GEE) model for multinomial responses were used.

Results: GEE models revealed that each month after the therapy start, the odds of lowering HBV DNA concentration increases by about 7 % on average (OR = 1.067, p = 0.0038) and the odds of the loss of HBV DNA by about 5 % (OR = 1.052, p 0.0001). Patients infected after 10 years of life have the odds of lower HBV concentration about 3 times (OR = 2.97) higher than the patients infected before 10 years of life, at any time point, on average. No associations was showed for modeling the normalization ALT activity.

Conclusion: Similarly to what has been reported in adults, ETV is effective treatment option for reduction of HBV DNA concentration in CHB children.

LBO-31

HeberNasvac: clinical development of a therapeutic vaccine

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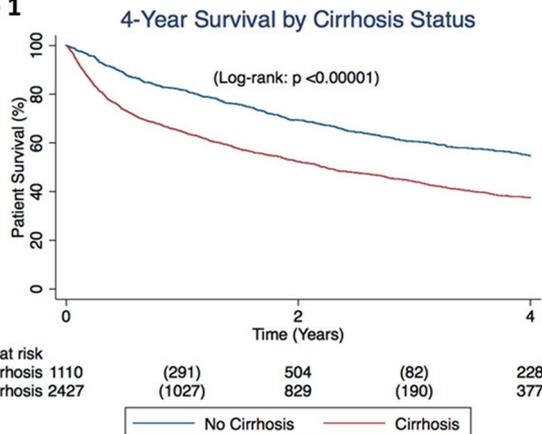
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The therapeutic vaccine candidate (TVC) HeberNasvac, is a novel formulation based on the 1:1 combination of the recombinant HBV surface and core virus like particle antigens (HBsAg and HBcAg). HeberNasvac is produced under good manufacturing practices at the production facilities of the Center for Genetic Engineering and Biotechnology. This vaccine candidate has been tested in different animal models in order to collect pharmacological evidences of their action mechanisms as well as the required safety preclinical data to support the product introduction in the clinical development. The present abstract summarizes the clinical trials conducted in healthy volunteers and in chronic hepatitis B patients. In general, HeberNasvac has proved to be safe when administered by IN or by parenteral routes. In addition, phase I–II and III results demonstrates that there is an antiviral response able to control the virus in most patients under treatment, resulting in a sustained effect when patients were followed for 6 months or a year after the end of treatment. These results support the introduction of HeberNasvac as a novel treatment for chronic hepatitis B (Fig. 1).

Table 1

Characteristic	Cirrhosis	No Cirrhosis	P value
Max tumor size (cm)	5.2	4.8	0.01
Unifocal tumor	46%	53%	<0.0001
BCLC stage C/D	43%	26%	<0.0001
Any treatment	81%	91%	<0.0001
Resection	14%	40%	<0.0001
Liver transplant	2.8%	0.8%	<0.0001
Liver-directed therapy	61%	47%	<0.0001

Figure 1



LBO-32

Portal vein embolization followed by right-side hemihepatectomy for hepatocellular carcinoma

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Background: Portal vein embolization (PVE) is useful to expand indications of major hepatectomy; however, its oncological effects are not fully understood. This study was conducted to confirm efficacies of preoperative PVE by a multi-institutional study.

Methods: Between 2000 and 2012, 510 hepatocellular carcinoma (HCC) patients undergoing right-side hemihepatectomy were enrolled [PVE group (n = 162) and non-PVE group (n = 348)]. To equalize background characteristics, one-to-one propensity-score matched analysis and multivariate analysis were performed. Short- and long-term outcomes were assessed.

Results: Propensity score-matched patients, 148 in each group, were selected. The percentage of resected liver volume before PVE was significantly greater in the PVE group (60.5 vs. 48.3 %, P < 0.001) but significantly decreased after PVE from 60.5 to 50.3 % (P < 0.001). The 5-year cumulative recurrence-free survival (RFS; 36.4 vs. 35.3 %) and overall survival (OS; 58.6 vs. 52.8 %) were comparable. The median duration to the first recurrence was significantly longer in the PVE group (15.3 vs. 8.0 months, P = 0.002). Extrahepatic recurrences were less common in the PVE group. Independent prognostic factors for RFS were morbidity [hazard ratio (HR), 1.56], multiple tumors (HR, 1.97), red cell concentrate administration (HR, 1.57), greater age (HR, 2.09), and massive portal invasion (HR, 2.33), whereas those for OS were morbidity (HR, 2.37),

multiple tumors (HR, 1.71), and massive venous invasion (HR, 3.49) (Fig. 1).

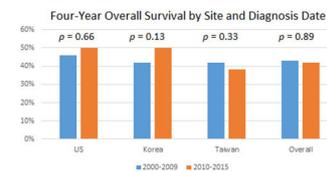
Conclusions: Even though HCC patients who underwent preoperative PVE and right-side hemihepatectomy had a significantly larger resected liver volume on admission, they have a comparable long-term outcome as patients with straight hepatectomy.

Table 1

Descriptor	2000-2009				2010-2015			
	Overall	US	Korea	Taiwan	Overall	US	Korea	Taiwan
Age (years)*	59.8	58.5	52.3	61.3	62.5	63.4	54.4	64.3
Cirrhosis*	73.8%	62.1%	85.0%	74.7%	63.9%	67.9%	80.9%	59.7%
MELD*	10.2	10.1	9.9	10.3	9.3	10.1	8.8	9.4
Symptomatic	26.5%	36.7%	44.5%	21.2%	24.8%	30.0%	41.1%	20.6%
Max tumor size (cm)*	4.7	4.9	6.4	4.3	5.3	4.8	6.1	5.2
Single tumor	56.4%	50.8%	64.6%	-	57.6%	48.7%	61.8%	-
BCLC stage C/D*	36.8%	19.2%	43.3%	40.0%	41.7%	26.1%	34.6%	44.9%
HCC treatment	83.6%	76.8%	89.0%	84.4%	82.2%	64.4%	91.9%	81.9%
Resection*	14.0%	30.0%	20.5%	9.2%	22.1%	22.6%	36.6%	18.8%
Percutaneous	9.1%	2.4%	10.6%	9.4%	7.6%	7.7%	7.7%	7.7%
TACE*	55.8%	46.5%	52.8%	58.5%	46.0%	45.2%	44.7%	46.3%
Sorafenib*	0.5%	1.9%	1.6%	0	3.5%	12.2%	4.1%	2.5%

*, p < 0.05 between the two time periods, overall. N/A, not available. BCLC, Barcelona clinic liver cancer. MELD, model of end-stage liver disease. TACE, transarterial chemoembolization.

Figure 1



LBO-33

The fate of recurred hepatocellular carcinoma after curative primary liver resection

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Background: Liver resection offers the best outcome among the treatment options of hepatocellular carcinoma (HCC) in Korea in which the patient with HCC could not get a chance of deceased donor transplantation due to organ shortage. Even after surgical resection, the majority of patients will develop intrahepatic recurrence of HCC. However the fate and associated prognostic factors of the recurred tumor has not been well established.

Methods: Between 2005 and 2011, a total of 959 patients who had intrahepatic recurrence after curative resection were reviewed.

Results: At primary resection, single HCC 912 (95.1 %) patients had HCC within Milan's criteria and post-operative AFP more than 40 ng/mL showed in 224 (24.9 %)/675 (75.1 %). On pathologic microscopic vascular invasion was 278 (35.5 %), major vessel involvement 292 (57.5 %), the worst Edmondson-Stein grade IV 106 (11.1 %), and multi-nodular type 190 (23.1 %). At the time of recurrence, 461 (48.6 %) patients showed recurrence within 10 month, 750 (81.8 %) patients had HCC within Milan's criteria. In multivariate analysis, the factors associated with OS after 1st recurrence were the Microvascular invasion (OR 2.01) and Recurrence within 10 month (OR 2.25). The 5-year OS rates after the 1st recurrence of patients with at least one of these factors (39.4 %) was poorer than those without these risk factors (77.5 %).

Conclusion: The pathologic microvascular invasion and early recurrence after primary liver resection were the important factors of OS rates after the recurred HCC after primary resection. Those factors would be guideline of decision for treatment of recurred HCC after primary resection.

LBO-34

Serial changes in M2BPGi levels as predictors of fibrosis and HCC in chronic hepatitis C

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Background: Mac-2-binding protein glycosylation isomer (M2BPGi) values were recently proposed as a noninvasive marker for liver fibrosis. The aim of this study was to evaluate the utility of serial measurement of M2BPGi through a treatment period as predictors of liver fibrosis and development of hepatocellular carcinoma (HCC).

Methods: Fifty-five patients with chronic hepatitis C who were treated with pegylated-interferon (PEG-IFN) and ribavirin (RBV) were included, and serum M2BPGi values were serially measured at pre-treatment, the end of treatment, and 24 weeks after completion of therapy.

Results: M2BPGi values transiently elevated at the end of treatment, and decreased at 24 weeks after completion of treatment in both patients with sustained virological response (SVR) and non-SVR. Pre-treatment levels of M2BPGi were significantly correlated with fibrosis, whereas this correlation became less significant in post-treatment M2BPGi, because M2BPGi decreased regardless of degree of fibrosis 24 weeks after the treatment especially in patients with SVR. On the other hand, pre- and post-treatment levels of M2BPGi in patients who developed HCC were significantly higher than those without HCC (pre-treatment, $p = 0.036$; post-treatment, $p = 0.0025$). ROC analysis revealed that pre-treatment M2BPGi was a useful predictive marker for advanced fibrosis (F3-4), and post-treatment M2BPGi 24 weeks after completion of treatment was a useful for predicting development of HCC with cutoff level of 2.2.

Conclusions: M2BPGi is useful to evaluate liver fibrosis at pre-treatment and to predict development of HCC at post-treatment. Screening by real-time monitoring of M2BPGi could be helpful for assessing HCC risk after anti-viral therapy.

LBO-35

Diagnosis value and correlation with immune regulatory factors of MSP in ACLF patients

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Objective: To observe the expression of macrophage stimulating protein level in acute-on-chronic liver failure patients, and explore the clinical significance and correlation with different immune regulatory factors of this cytokine.

Method: Double antigen sandwich method to detect MSP in peripheral blood in 45 cases diagnosed with ACLF and 32 cases with chronic hepatitis B (CHB), the healthy peripheral blood serum as

control. Compared the expression of MSP in different prognosis of patients with ACLF, detect the liver function, hepatitis B virus (HBV), peripheral blood CD4⁺ interferon γ ⁺, CD4⁺ interleukin4⁺, CD4⁺ IL-17⁺, CD4⁺ CD25⁺ FOXP3⁺ in different groups. Analysis of variance between different groups.

Results: MSP level was significantly higher in ACLF patients serum 1.65 ± 0.46 ng/mL than that in CHB group 1.43 ± 0.32 ng/mL and healthy control 1.23 ± 0.21 ng/mL $P < 0.01$. CHB group and healthy control group had no statistical difference. ACLF survival group (1.82 ± 0.32 ng/m) ACLF death group 1.17 ± 0.22 ng/m, there was significant difference between the two groups $P = 0.042$. Lymphocyte Th2, Th17 cells in the group of ACLF were higher than the other two groups $P < 0.01$, while about Th1, Treg cells, there was no different compare with the two groups, MSP level and Th2, Th17, Th17/Treg lymphocyte number changes were positively correlated $r = 0.386, 0.644, 0.605, P = 0.032, 0.000, 0.000$.

Conclusion: MSP was involved in the progress of ACLF, there is a certain relationship between the level change of MSP, with the clinical outcomes and cellular immune imbalance in ACLF patients, MSP is very important to the future clinical diagnosis and treatment of acute-on-chronic liver failure.

LBO-36

The incidence, outcomes and cost of drug-induced liver injury from a national database in Thailand

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Background: Toxic liver diseases are contributed mostly by drug-induced liver injury (DILI). The Universal Coverage Scheme covers the health expense of 48 million of Thai population. We assessed incidence, presentation, outcomes and economic burden of DILI including the associated factor of mortality.

Method: We retrieved the data from the 2009–2013 Nationwide Hospital Admission Data using the ICD-10 2010 code of toxic liver diseases (K71). Factors associated with mortality were analyzed with log-rank test, univariate and multiple cox regression analysis.

Results: A total of 6,516 admissions were due to toxic liver diseases. The most common type was acute hepatitis (33.5 %). The in-hospital and 90-day mortality rates were 3.2 and 19.9 %, which were lower than overall liver diseases (6.8 and 29.2 %) ($P < 0.001$). Toxic liver disease with cirrhosis yielded the highest in-hospital and 90-day mortality rate (15.8 and 47.4 %). The independent factors associated with mortality were acetaminophen (hazard ratio [HR] 0.24, 95 % confidence interval [CI] 0.13–0.42), cirrhosis (HR 2.72, 95 % CI 2.32–3.19), age >60 years (HR 2.16, 95 % CI 1.96–2.38), HIV (HR 2.11, 95 % CI 1.88–2.36), CKD (HR 1.59, 95 % CI 1.33–1.90), COPD/bronchiectasis (HR 1.55, 95 % CI 1.17–2.04), malnutrition (HR 1.43, 95 % CI 1.10–1.86), and being male (HR 1.31, 95 % CI 1.21–1.43). The etiology of non-acetaminophen, cirrhosis and elderly yielded significant impact on long-term survival ($P < 0.001$). The three most common causes were acetaminophen (35.0 %), anti-mycobacterial (26.7 %) and rifamycin (8.0 %).

Conclusion: The mortality rate of toxic liver diseases is significantly increased in non-acetaminophen taking, cirrhosis, elderly, having concomitant diseases and male. Acetaminophen and anti-tuberculosis drugs are the most common causes.

LBO-037

Amatoxin poisoning: interplay of renal function, lactate values, iv hydration and silibinin

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Background: Italian PCCs: 10 % AP mortality, but 6 transplants/3 deaths, October 2012. India, Vietnam, ALFSG, Australia: 50 % death/liver transplant. Rising LA/ORF preceded FHF: Australian, ALFSG poor outcomes; 8 California deaths since 2011.

Methods: Reviewed labs, orders: India/Vietnam APs 2011/2014; US SIL APs 2007–2015; 14 CA SIL enrolled transplant unit APs 2007–09.

Results: India/Vietnam pts without AH: ORF, FHF/death. Survivors received AH, avoided ORF. IV fluid discontinued 3 transplant unit arrivals; rapid LA rise, ORF/FHF. 2 died, 1 transplant. 11 recovering with SIL/ongoing IV hydration, had normal LA. Poor outcomes all US SIL ORF pts; full recoveries when ORF avoided.

Conclusions: Presentation hypoperfusion type A LA typical; corrects with AH. Type B FHF occurs later. Serial LA earliest, most sensitive AP prognostic indicator. Uncorrected LA: inadequate IV hydration; poor outcomes. LA correction: favorable prognosis; requires sustained AH. Italian PCCs emphasize AH/brisk urine output. Minimizing transit time protects kidneys. Mild-moderate ingestions recover with AH alone. Enormous unmet need antidote AP FHF. SIL licensed in Europe, not universally used. Success/failure not predictable/understood as no prospective trial until NOW: If ORF avoided, INR improvement by 36th h of SIL infusion heralds FHF reversal/rapid recovery in severe poisonings. SIL success requires AH. Failure to provide AH/late presentation leads to ATN/ORF, hypoperfusion/amatoxin mediated AKI. ORF compresses AP timeline; accelerates hepatic injury pace/severity. Small ingestions suffer poor outcomes if urine output not rapidly restored. Poor outcomes: kidneys fail BEFORE liver is Root cause of excessive AP mortality: failure to provide AH. Fluid management determines outcomes.

LBO-38

A novel mutation of ATP8B1 gene in young patient with familial intrahepatic cholestasis

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Abstract: A novel mutation in ATP8B1 gene is described in 18 years old patient with familial intrahepatic cholestasis with late onset of symptoms in age of 17. Elevated AP, ALT with normal GGT, recurrent jaundice and itching were presented. BRIC is suspected due to intermittent course of disease and absence of significant findings in liver biopsy.

Materials and methods: Liver biopsy with HE and trichrome stain was performed. Viral hepatitis A, E, B, C, EBV, and CMV infection, drugs or alcohol exposure, Wilson's disease, A1ATD, as well as PSC and PBC, autoimmune hepatitis were excluded. Partial analysis of ATP8B1 (3, 4, 5, 6–7, 8–9 exons), ABCB11 (p. Asp482Gly fragment), ABCB4 (9, 16–17, 18, 20 exons) by direct automatic sequential assay were analyzed.

Results: A polymorphic variant c.504C>T (rs1202283) in ABCB4 gene (9 exon) was revealed and unsubscribed mutation in ATP8B1 gene: duplication of 1 nucleotide c.534dupT (heterozygous) leading to stop-codon p.Glu179* and premature ending of protein synthesis. Whole-genome assay of ATP8B1 gene showed no additional mutations. Liver biopsy demonstrated bilirubinostasis in acinus zone 2 and 3, slight cholangiopathy and no signs of fibrosis or inflammation.

Conclusion: A novel mutation in ATP8B1 gene (stop-codon p.Glu179* due to duplication c.534dupT in 9 exon) leading to forming of stop-codon p.Glu179* is described in 18 years old patient with late onset of familial intrahepatic cholestasis and family history of AIH in father. Being heterozygous, this could explain late onset and benign course of disease without significant liver fibrosis and represents either BRIC or both PFIC1.

LBO-39

A replicon-baculovirus model for efficient packaging of HEV RNA and production of infectious virions

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Hepatitis E virus (HEV) is an emerging RNA virus that causes acute and chronic liver disease with a global mortality rate of about 2 %. Despite milestone developments in understanding of HEV biology, there is still lack of a robust culture system or animal model. Therefore, in a novel approach, two recombinant-baculoviruses (vBac-ORF2 and vBac-ORF3) that could overexpress HEV ORF2 (structural/capsid) and ORF3 (nonstructural/regulatory) proteins, respectively were constructed. The established HEV-SAR55 (genotype 1) replicon that contained GFP gene, in place of ORF2/ORF3 sequences was in vitro transcribed, and GFP production in RNA transfected S10-3 cells was scored by FACS. Enhanced infectivity, if any, of nascent virions produced by exogenously-supplied ORF2 and viral RNA by co-expression of ORF3 was tested on naive HepG2 cells. Co-transduction with vBac-ORF2/vBac-ORF3 (108 pfu/microL) produced high amounts of native ORF2/ORF3 in approximately 60 % of S10-3 cells, determined by immunofluorescence microscopy and Western analysis. FACS analysis showed about 9 % GFP positivity of S10-3 cells on day 6 post-transfection (i.e., day 5 post-transduction). Further, FACS scoring indicated that lysates from S10-3 cultures receiving the RNA plus vBac-ORF2 were capable of producing HEV particles with about 4 % infectivity in HepG2 cells. However, lysates of cultures co-transduced with vBac-ORF3, were found to further enhance virion infectivity by approximately 17 %. This supported a previously proposed role of ORF3 as a minor-structural protein in HEV virion assembly and infectivity. In conclusion, our present model for efficient genomic RNA packaging and production of infectious virions offers a valuable tool to study various aspects of HEV molecular biology, in vitro.

LBO-40

MiR-B-Index: a biomarker to identify the immune control of HBV infection in CHB pts treated with NA

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Background and aims: Increasing evidences indicate that Nucleos(t)ide-analogues (NA) can be safely discontinued in a proportion of chronic-hepatitis-B (CHB) patients (pts). However, markers of the achieved immune-control are missing. We studied, in NA-treated pts, the dynamics of a serum microRNA-signature (MiR-B-Index, MBI) associated with the natural and therapy-induced immune-control of HBV-infection.

Patients and methods: MBI [(RT-PCR for miR-122-5p/miR-99a-5p/miR-192-5p/miR-335-5p/miR-126-3p/miR-320a); -1.7 cut-off for inactive-infection] was measured at baseline (BL) and end-of-follow-up-(EOF)/end-of-therapy(EOT) in 194 pts [37/157 HBeAg pos/neg; 153 males; median-age 55 years] treated with NA (median 65 months, 12/181 m).

Results: MBI changed significantly from BL to EOF: median -7.07 ($-18.42/9.37$) vs 0.040 ($-13.08/14.17$), $p < 0.001$. BL-MBI correlated (multivariate-analysis) with HBsAg-Log-IU/mL ($p < 0.001$), HBV-DNA-Log-IU/mL ($p = 0.009$) and ALT ($p = 0.045$). EOF-MBI independently correlated with EOF-Log-HBsAg ($p < 0.001$), and ALT ($p = 0.014$). Both EOF-MBI and MBI- Δ -variations were higher in pts with >1 Log-HBsAg decline [3.49 $-8.92/12.05$] vs -1.58 ($-13.08/14.17$), $p < 0.001$ and 11.59 ($2.00/22.65$) vs 4.24 ($-3.78/15.95$), $p < 0.001$, respectively]. The MBI- Δ -variation was independently associated with BL-Log-HBsAg ($p < 0.001$), EOF-MBI ($p < 0.001$), HBsAg- Δ -variation ($p = 0.002$) and treatment-duration ($p = 0.003$). 19 patients stopped NA; 9 after HBeAg/anti-HBe-seroconversion: EOT-MBI was -9.33 ($-12.76/-4.55$) in 4 pts with HBeAg-recurrence and 6.43 ($-1.70/10.11$) in the 5 carriers with sustained-virologic-response (SVR). In 10 HBeAg- with HBsAg-loss/ <50 IU/ml EOT-MBI was 6.14 ($-2.57/14.01$). MBI was ≥ -1.7 at EOT in all but one SVR and at EOF in 123/194 (63.4 %) pts:68 (55.3 %) and 41 (33.3 %) of them had HBsAg levels ≤ 400 and ≤ 100 IU/mL respectively.

Conclusions: These results suggest that MBI combined with qt-HBsAg could identify the sustained switch of CHB to inactive HBV-infection during NA-treatment.

Late Breaking Poster Presentation

LBP-001

Subcellular localization of cyclin G1 during mitosis in JFH1-hmAG infected Huh7.5 cells

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Background: Cyclin G1, a miR-122 target, was identified as a novel host cofactor for HCV replication. JFH1-hmAG was an infectious

monocistronic reporter virus expressing Azami-Green. The exact roles of Cyclin G1 in HCV replication is still unknown. The aim of this study is to investigate the subcellular localization of Cyclin G1 during mitosis in JFH1-hmAG infected Huh7.5 cells.

Methods: Huh 7.5 cells were cultivated using routine method. An infectious reporter virus, JFH1-hmAG, was inoculated into Huh 7.5 cells. Intracellular staining was performed using routine method. Cells were stained with anti-Cyclin G1 antibody (red fluorescence) and were counterstained with DAPI (blue fluorescence). Confocal imaging was performed on a Leica TCS SP5 II confocal microscope.

Results: Cyclin G1 staining (red fluorescence) was seen throughout mitosis. During prophase, the localization of Cyclin G1 is completely in the nucleus. However, during metaphase, anaphase and telophase, Cyclin G1 fluorescence showing diffuse labelling in the cytoplasm of the cell. Particularly during anaphase, Cyclin G1 aggregated predominantly peripherally close to the condensed chromosomes. Co-localization of Cyclin G1(red fluorescence) with NS5A(green fluorescence) in the cytoplasm as yellow fluorescence was found during metaphase, anaphase and telophase.

Conclusions: We showed for the first time that Cyclin G1 is transiently distributed from the nucleus to the cytoplasm during mitosis. Cyclin G1 co-localized with NS5A during the metaphase, anaphase and telophase of the mitosis. This work was supported in part by the National Natural Science Foundation of China (30800974, 81271845) and Tianjin Municipal Health Bureau of science and Technology Fund (2012KR02, 12KG118).

LBP-002

In vitro characterization and drug sensitivity analysis for HCV NS5A resistance-associated variants

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Backgrounds: The direct antiviral agents for hepatitis C virus (HCV) is known to have potent anti-HCV effects, whereas it may provoke the resistance-associated variants (RAVs). RAVs against NS5A inhibitors are often detected in treatment naïve patients and persist after treatment. In this study, we assessed the characteristics of these RAVs and explored the efficacious anti-HCV reagents by using recombinant HCV with NS5A of Con1 (genotype 1b).

Methods: We replaced the NS5A of JFH-1 with that of Con1 (JFH1/5ACon1) and introduced known NS5A inhibitor-resistance mutations (L31M, L31V, L31I and Y93H). Full-length HCV RNAs of these strains were transfected into Huh-7.5.1 cells and HCV core antigen and infectivity titers were measured. Susceptibilities against anti-HCV reagents were also investigated.

Results: The RAVs with Y93H exhibited high extracellular core Ag levels and infectivity titers. In the analysis for susceptibility to NS5A inhibitors (DCV and LDV), strains with any single mutation showed the mild to moderate resistance, while strains with double mutations of both L31 and Y93 showed severe resistance. These strains with mutations exhibited similar level susceptibility to IFN α , IFN λ 1, IFN λ 3 or RBV. Interestingly, the strains with Y93H mutation were more sensitive to protease inhibitors (ASV and SMV) in comparison with JFH-1/5ACon1.

Conclusions: In the in vitro analysis, we indicated that Y93H mutation could enhance the infectious virus production suggesting advantages in propagation of RAVs with this mutation. However, these RAVs were revealed to be susceptible to protease inhibitors, and the therapeutic regimen with these reagents will be promising to eradicate these RAVs.

LBP-003

T cell subpopulation predict the occurrence and prognosis of cryoglobulinemia in chronic hepatitis C

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Background: Cellular immune responses play an important role in the immune-pathogenesis of hepatitis c virus (HCV) infection. Essential mixed cryoglobulinemia with dermatologic, neurologic, renal, and rheumatologic complications is linked to HCV infection.

Objective: To assess the role of T cell subpopulation in prediction of occurrence and prognosis of cryoglobulinemia in chronic hepatitis C patients.

Methods: Hundred HCV positive patients were enrolled. They were subdivided into 2 groups: Group A: HCV infected Patients with cryoglobulins positive antibodies. Group B: HCV Patients with cryoglobulins negative antibodies. All patients were subjected to CBC, ESR, serum urea and creatinine, RBS, liver function tests, pelvi-abdominal US, measurement of Cryoglobulins antibodies and measurement of CD4, CD8, and CD4 CD8 ratio.

Results: Frequency of cryoglobulinemia between HCV patients was 19 % and symptomatic cryoglobulinemia was 6 %; females (68 %) were more affected than in males (32 %). More than 85.5 % of symptomatic patients have purpura and arthralgia, 71.4 % have paresthesias, 28.5 % have Livedo reticularis, 14.2 % have chronic lymphocytic leukaemia. The mean age of patients with cryoglobulin was 59.2 Year and mean duration of infection of HCV was 24 years. All patients with cryoglobulins were cirrhotic, with significant correlation between cryoglobulinemia and elevated AST, ALT and impaired synthetic and excretory functions of the liver. CD4 and CD8 were lower in chronic HCV patients with cryoglobulinemia than without cryoglobulinemia.

Conclusion: T cell subpopulation can predict the occurrence and prognosis of cryoglobulinemia in chronic HCV patients. Cryoglobulinemia is linked to liver cirrhosis and impairment of synthetic and excretory functions of the liver.

LBP-004

Three important disorders Regarding hepatitis C in the middle east

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Introduction: Hepatitis C, one of the five well-known types of viral hepatitis, is transmitted through blood and body fluids. HCV, as one of the widespread infections all over the world, can cause chronic diseases. Based on WHO estimates, 3 % of people are infected with this virus. Reviewing the literature, this study intends to analysis the three important disorders related to HCV in the Middle East.

Materials and methods: In this literature review, all of international statistics about HCV and three related disorders such as inherited coagulation, Thalassemia and undergo Hemodialysis patients, were collected from international and Iranian journals, papers and communities. In Iran, prevalence of HCV infection was evaluated in eight provinces <45 % of Iran's total population >. Regarding superimposed HCV infection in patients with Thalassemia, cardiovascular involvement accounted for most of the cases of mortality. However, recently conducted studies revealed the liver diseases as the main cause of death.

Results: Overall estimate of HCV infection among patients with inherited coagulation disorders was 48.07 % in Iran and 48.27 % in all the EMRO countries. Epidemiology of HCV in Thalassemia patients is as follows: Iran 18 %, Bahrain 32 %, Saudi Arabia 63 %, Kuwait 33 %, Iraq 65 %, Jordan 40 % and Egypt 69 %. Epidemiology of HCV in patients who need to undergo Hemodialysis is as follows: In Iran 16 %, Bahrain 9 %, Kuwait 45 %, UAE 24 %, Oman 27 %, Iraq 7 %, Saudi Arabia 63 %, Egypt 48 % and Qatar 45 %.

Conclusions: The patients with HCV infection are growing and prevention proceedings are more important than past.

LBP-005

IL28B polymorphism as in chronic hepatitis C genotype 3

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Background: Genetic variation in the interleukin 28B (IL28B) gene has been associated with response to Interferon and Ribavirin therapy in hepatitis C virus genotype 1-infected patients; there is limited data for HCV patients with genotype 3. We evaluated the effects of IL28B polymorphisms on response to treatment with IFN+ Riba in patients with HCV genotype 3.

Material and methods: This cross-sectional study was conducted from July 2013 to June 2014. IL28B host genotypes (CC, CT and TT) were analyzed in 184 patients of chronic hepatitis C genotype 3.

Results: The frequencies of the IL28B genotypes were as follows: CC, 24.5 %; CT, 60.3 %; and TT, 15.2 %; Non-CC genotype was most frequently found in HCV genotype 3 non-responders to IFN and RBV, irrespective of their gender and genotype subtype status.

Conclusion: An IL28B polymorphism (Non-CC genotype) was associated with Virological non-Response in patients infected with genotype 3 HCV who did not achieve RVR. Analysis of IL28B genotype might be used to guide treatment for these patients.

LBP-006

Relationship between IL28B gene polymorphism and antiviral efficacy in children with CHC

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Background and aims: To explore the effect of single nucleotide polymorphisms of interleukin (IL)-28B, rs12979860 on the antiviral treatment response in children with chronic hepatitis C(CHC).

Methods: 129 children with chronic hepatitis C were enrolled, who were treated with alfa-interferon 2b in combination with ribavirin(RBV) for 48 weeks, which were followed up for 24 weeks after the cessation of therapy. The treatment efficiency was evaluated with sustained virological response(SVR),and the relationship between IL-28B polymorphism rs12979860 and SVR was analyzed.

Results: Among 129 patients, 104 patients (80.6 %) was IL-28B rs12979860 C/C, 25 patients (19.4 %) IL-28B rs12979860 C/T. There was no statistics difference in sex, hepatitis C virus (HCV) genotype, aminotransferases(ALT) level and baseline viral load between the patients with IL-28B rs12979860 C/C and IL-28B rs12979860 C/T ($p < 0.05$). The rates of SVR were higher in patients with IL-28B rs12979860 C/C than C/T (99.0 vs 80 %, $p = 0.002$). There were no statistics difference in SVR between male and female (98.0 vs 94.3 %, $p = 0.456$), HCV genotype 2a and those with HCV genotype 1b (98.6 vs 93.0 %, $p = 0.160$), high or low ALT level as well as baseline viral load.

Conclusions: Compared with HCV viral genotype, ALT level and baseline viral load, IL-28B rs12979860 is more valuable for predicting antiviral efficacy in children with CHC, IL-28B rs12979860 C/C is an independently predictive factor associated with higher SVR in children and it can provide a reliable data for individual therapy.

LBP-007

Blood group and IL-28B genotype in PEG-IFN- α and RIBAVIRIN therapy for HCV-1 and liver fibrosis

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Aim: to evaluate the prognostic significance of the blood group and interleukin-28B(IL-28B) polymorphism in achievement of sustained virologic response (SVR) on antiviral therapy with Pegylated interferon alpha2 (peg-IFN α -2) and ribavirin in patients with chronic HCV-1 and these factors influence on liver fibrosis progression in case of nonresponders. 146 HCV-1 patients were examined. Clinical and laboratory data, qualitative and quantitative analysis of HCV RNA, blood group affiliation, IL-28B polymorphism, liver fibrosis severity by liver biopsy and fibroscan were performed. Liver fibrosis dynamics was assessed in 40 patients with SVR. The control group were 20 patients with out antiviral treatment.

Results: SVR was obtained in 56.8 %. Combination of IL-28B polymorphism and blood group significantly influenced on SVR ($p = 0.00024$). Virologic response was observed on combination of O(I) blood group and C/C and T/T IL-28B genotypes, A(II) blood group with C/T and T/T, B(III) blood group- with T/G in 100, 88.2, 94.4 %, respectively. Virologic response in patients with A (II) blood group and one—or two-nucleotide substitution in rs 8099917 gene IL-28B (genotype TG or GG) was 0 %, and fibrosis assessment showed progression in all patients of this group. B(III) blood group patients had SVR-83.8 %.

Conclusions: Determination of the blood group in combination with the IL-28B polymorphism allows to predict SVR on the treatment of HCV-1 by peg-IFN α -2 + RBV.

LBP-008

Distribution of resistance-associated variants (RAVs) in HCV genotypes (GT) 1a and 1b populations

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Background: Antiviral drugs in combination with interferon and ribavirin significantly improve virologic response rate in patients with GT1 chronic hepatitis C. One of the most important considerations is the potential development of RAVs. Timely detection and reporting of RAVs and HCV GTs is critical for drug regimen and can minimize the development of resistance to antiviral drugs.

Methods: We used a newly developed automated Next Generation Sequencing (NGS)-based integrated workflow, comprised of a robotic liquid handling system, kits for RNA extraction and library preparation (Sentosa SQ HCV Genotyping Assay), Ion Torrent based deep sequencing system and bioinformatics analysis and reporting software. The data reports on GTs 1a and 1b include 136 known RAVs in NS3, NS5A and NS5B genes. However, the system does not make direct treatment recommendations, which are left to investigators.

Results: This study included 56 GT1a and 54 GT1b samples. 52.7 % (58/110) of HCV strains were carrying 1 or multiple RAVs in 23 positions in all target genes. An unequal distribution of 4 mutations across the GT1 subtypes was observed. Frequency of Q80K mutation (NS3) was 25 % (14/56) in GT1a and 1.9 % (1/56) in GT1b. While mutations Q54H and Y93H (NS5A) were prevalent in GT1b: 42.6 % (23/56) and 18.5 % (10/56) respectively. Frequency of Q54H in GT1a population was 0 % (0/56) and Y93H—1.8 % (1/56). Mutation V499A in NS5B gene was also prevalent in GT1b: 0 % (0/56) in GT1a and 25.9 % (14/56) in GT1b populations.

Conclusion: Simultaneous RAVs detection and GTs determination by one diagnostics tool provides complete, essential and important information for HCV treatment approaches.

LBP-009

Eosinophilic enterocolitis in a patient with chronic hepatitis C

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Background: Eosinophilic enterocolitis is a relative uncommon disease characterized by accumulation of eosinophils in the intestine, causing clinical symptoms including abdominal pain, vomiting, diarrhea, weight loss, etc. Some literatures had demonstrated the association between eosinophilic enterocolitis and chronic hepatitis C with peg-interferon therapy. We presented one HCV patient who was diagnosed eosinophilic enterocolitis.

A 35-year-old man had chronic hepatitis C. He complained 1 month of abdominal pain, anorexia, diarrhea and weight loss. The serum WBC was 15,100/ μ L with 29 % of eosinophils. Esophagogastroduodenoscopy revealed duodenitis. Colonoscopy showed hyperemic

mucosal swelling at terminal ileum, cecum and ascending colon. The pathological examination confirmed eosinophils infiltration (picture 1) in terminal ileum and ascending colon. In addition, the abdominal CT displayed diffuse wall thickening at jejunum, ileum, colon with some ascites (picture 2). The WBC of his ascites was 1000/cmm with 91 % of eosinophils. We prescribed corticosteroid and the symptoms improved. Finally the patient was completely free of symptom.

Discussions: The pathogenesis eosinophilic enterocolitis and chronic hepatitis C was not well established. Peg-interferon was considered to be one of the possible causative factors in patients during anti-HCV treatment, by activating cytokine IL-5 and chemokine eotaxin. In our article, the patient didn't receive antiviral therapy but still had eosinophilic enterocolitis. Is there any association between these diseases or just co-existence? Some physicians observed the eosinophils infiltration in liver of HCV patients. Co-existence of eosinophilic granulomatosis and HIV, HCV has also been described. To determine the mechanism of these disorders, further analysis of larger data is required.

LBP-010

The excellent outcome of interferon-based hepatitis C treatment in Taiwanese incarcerated patients

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Background: The prevalence of hepatitis C virus (HCV) infection is approximately 40 % in Taiwan's inmates, and most of them are drug abuser. These patients have not receive interferon-based therapy after national health insurance coverage to inmates since January, 2013. Is the outcome of these incarcerated patients better than that of community ones?

Methods: This study was a retrospective study. We compare CHC patients in Taichung Prison, who underwent interferon-based treatment between January 1, 2013 and December 1, 2015, with those treated in CMUH in the past 10 years. Information obtained from the patient record included patient medical history, psychiatric history, substance abuse history, HCV genotype, hemogram, liver function tests, and side effects during treatment.

Results: There were 99 chronic hepatitis C patients (CHC) undergoing interferon therapy in the jail. Incarcerated patients are mostly male, and drug abuser and younger than community patients. In addition, patients with genotype 1 or 6 are predominant. A sustained viral response (SVR) was achieved 91 (91.9 %) incarcerated patients, compared to 925 (75.3 %) community patients ($p = 0.0002$). As for genotype 1 or 6 group, a SVR was achieved in 37 (94.9 %) prisoners treated in 24 weeks, and 27 (84.4 %) prisoners treated in 48 weeks, compared to 167 (65 %) and 338 (67.2 %) community patients treated in the same interval, respectively ($p = 0.0002$, and 0.043). These two groups are almost no difference in side effects, except anemia and hypothyroidism.

Conclusions: These excellent results might be due to their good drug compliance, male gender and the younger age.

LBP-011

Cost-effectiveness of IFN + RBV α 2b for hepatitis C: results based on the interferon database

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Background: Hepatitis C is a costly disease to treat despite the efficacy of antiviral drugs in infected patients. Numerous global studies have investigated the economic value of hepatitis C treatments; however, the disease variation and population characteristics differ by country, which may affect outcome data. Real world data may be useful for evaluating the cost-effectiveness of hepatitis C drugs, but few studies currently exist.

Methods: The cost-effectiveness of peginterferon plus ribavirin (IFN + RBVType α 2b [48 weeks]) was studied using the Japanese interferon database. The search included individuals with chronic hepatitis C genotypes 1 and 2 registered between 2009 and 2013 (chronic hepatitis C: $n = 9354$, compensated cirrhosis: $n = 270$). Cost-effectiveness was estimated using a Markov-based method called the MODelling the Natural histoRY and Cost-effectiveness of Hepatitis (MONARCH) model. Drug costs were determined from package inserts and the guidelines.

Results: The cumulative 10-year cost-effectiveness of IFN + RBV-Type α 2b (48 weeks) treatment is estimated to increase quality-adjusted life-years (QALYs) by 6.1 years at a cost of 14.6 million JPY/119,854 USD in Japanese patients with hepatitis C genotypes 1 and 2. The cost-effectiveness ratio is about 2.4 million JPY/19,702 USD per QALY.

Conclusions: This study verified the usefulness of real world data to estimate cost-effectiveness of IFN + RBVType α 2b in Japanese patients with hepatitis C genotypes 1 and 2. Additional studies considering subgroup analysis using other information in the database may clarify the cost-effectiveness details.

LBP-012

Is rapid virological response (RVR) significant in the present era in the treatment of Hepatitis C

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Introduction: Treatment of hepatitis C has dramatically improved over the last decade. Unlike other chronic infections, significant number of patients can be cured. RVR is the best predictor of response to PEGylated interferon/Ribavirin. We want to find whether the same can be replicated in patients treated with sofosbuvir or not. **Methods:** We prospectively evaluated patients with hepatitis C taking treatment from LTMGH, both cirrhotic and non-cirrhotic, treatment naive and exposed, Genotype 1 and Genotype 3. 25 patients were started on Sofosbuvir \pm PEGylated Interferon \pm Ribavirin (SPR) for 12 weeks and 33 patients were treated with PEGylated Interferon \pm Ribavirin (PR) according to genotype. Number of patients who achieved RVR and ETR (End of Treatment Response) in both the treatment groups was calculated and appropriate statistical tests applied (Fig. 1).

Results: in the SPR treatment group, out of 25 patients all achieved RVR (n=25,100 %). In the PR treatment group, RVR achievement was 75 % (n=24). Out of those who did not achieve RVR (n=8), 5 patients achieved ETR. All 25 patients in SPR treatment group achieved ETR. RVR in SPR group is higher than PR group in GT 1 (100 vs 55 %) and GT 3 (100 vs 83 %).

Conclusion: All patients who achieved ETR had cleared HCV RNA at 4 weeks in the Sofosbuvir group as compared to PR group, irrespective of their genotype. Testing for HCV RNA at 4 weeks can be skipped in the new era of directly acting antivirals, thus making HCV treatment more cost effective.

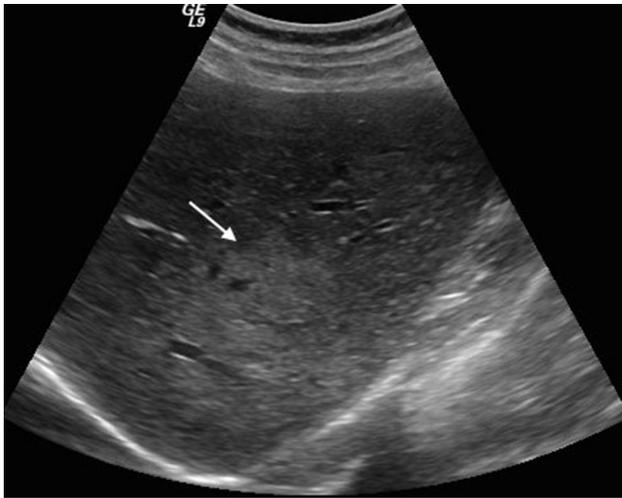


Fig. 1

LBP-013

The use of silymarin in chronic hepatitis C infection: updated systematic review and meta-analysis

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Significance: The current standard of care for HCV infection is expensive and unavailable in many countries. In vitro, silymarin can inhibit HCV replication and has anti-inflammatory and immunomodulatory actions. Clinical studies to evaluate this potential have inconsistent results or had low methodological qualities. The objective of this study is to assess the effects of silymarin in patients with chronic HCV infection using meta-analyses of available RCTs with good methodological quality.

Methodology: Manual and electronic searches through The Cochrane Central Register of Controlled Trials, MEDLINE, and PUBMED were done. RCTs with a quality scale of A-B that performed in vivo evaluation of the effects of silymarin in adults with HCV infection were included. Each study was independently appraised by 2 reviewers with regards to methods of minimizing selection bias, performance bias, exclusion bias, and detection bias. Discrepancies were resolved by a third reviewer. Data were analyzed using RevMan 5, and the Forest plots generated were interpreted accordingly. Chi

square test was used to test for heterogeneity and a p-value of 0.05 was considered significant.

Results: 13 RCTs were identified. Of these, 6 were excluded, 2 could not be retrieved, and 1 lacked data on HCV patients. 4 studies were included in the review. Oral silymarin preparations did not show statistically significant benefits in achieving SVR, decreasing viral load, and improvement of other liver parameters in patients with chronic HCV infection.

Conclusion: There is still no evidence to support the routine use of silymarin in the treatment of chronic HCV infection.

LBP-014

Correlation of virological and clinical profile of patients with BC-coinfection in Pakistan

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Objective: to find out and correlate the virological and clinical profile in patients with chronic hepatitis B and C co-infection.

Materials and methods: This observational, descriptive and cross-sectional study was conducted from July 2010 to June 2014. All patients with HBsAg and Anti-HCV Reactive by ELISA for more than 6 months were included in the study. Following investigations were carried out in these patients: Serum ALT, HBeAg, anti-HBe, HBV DNA PCR, HCV RNA PCR, and abdominal ultrasound.

Results: A total of 130 patients were studied, out of which 81 (62.3 %) were males. Mean age of patients was 40.52 years. Majority of patients belonged to age-group of 21–30 years. Mean serum ALT of patients was 83.69 U/L. Majority of patients belonged to ALT-Group of 41–80 U/L. Hepatitis C Virus was the dominant virus in 53 % of patients. Chronic Hepatitis was the dominant clinical profile in 73 % of patients.

Conclusion: Hepatitis C Virus is the dominant virus in our patients with BC co-infection. There is no statistically significant association between virological and clinical profile of these patients.

LBP-015

Pretreatment determinants of early virologic response in Sofosbuvir treated patients

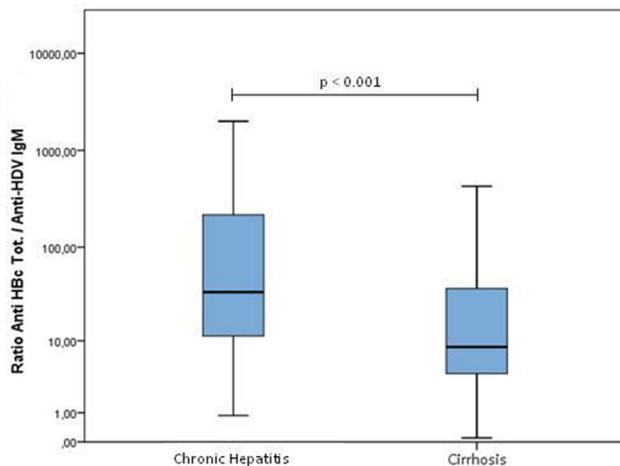
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Introduction: Sofosbuvir has been found to be highly effective in the treatment of HCV. Here we report the impact of pretreatment factors that may determine negative PCR at 2 weeks in HCV genotype 3 patients treated with Sofosbuvir and Ribavirin.

Results: PCR was checked at 2 weeks after initiation of Sofosbuvir and Ribavirin. Out of 252 patients, PCR was negative in 220 (87 %) patients and still positive in 32 (13 %) patients. Out of treatment and hepatic status, IL28B genotypes and viral load advanced hepatic fibrosis and non-CC IL28B genotypes were associated with delayed virologic response at 2 weeks. It needs to be determined if these factors can be used a predictors of SVR12. RESIP is an ongoing study and updated results will be presented in the meeting (Fig. 1).

Fig. 1



Conclusion: Advanced hepatic fibrosis and non-CC IL28B genotypes are associated with negative virologic response at 2 weeks of initiation of Sofosbuvir and Ribavirin.

LBP-016

Sofosbuvir-including regimens against real-world Japanese patients with HCV GT-1 and GT-2

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Background and aims: Interferon-free regimens are now available for the treatment of HCV GT-1 and GT-2 in Japan. We reported that the results of sofosbuvir-including regimens against real-world patients at the urban university hospital in Japan.

Materials and methods: We commenced to treat 169 HCV GT-1 or 99 GT-2 patients with fixed-dose combination of ledipasvir and sofosbuvir for 12 weeks or sofosbuvir plus weight-based ribavirin for 12 weeks, respectively.

Results: SVR rates at 12 weeks were 100 % and 80 % in HCV GT-1 and GT-2-infected patients, respectively, although the number of patients who have finished treatments was small at present. In HCV GT-2 patients, one stopped treatment due to adverse events and one was lost to follow up.

Conclusion: Interferon-free treatment with sofosbuvir seems safety and effective for Japanese patients infected with HCV GT-1 and GT-2. Now we consider interferon-free treatment with sofosbuvir as first choice of treatment regimens for HCV-infected patients in Japan.

LBP-017

Transmembrane protein 2 inhibits hepatitis B virus infection in L02 cells

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Background: Transmembrane protein 2 (TMEM2) belongs to the transmembrane protein superfamily. So far, the complete understanding of TMEM2 function remains elusive. In our previous study, we showed that the expression of transmembrane protein 2 (TMEM2) was down-regulated in the liver tissue of patient with HBV infection and HepG2.2.15 cells. Now we aim to investigate the function of TMEM2 in HBV infection in L02 cells.

Methods: Stably overexpressing TMEM2 cell lines (L02 TMEM2) were established using lentivirus vectors. Western blot (WB) and real-time quantitative PCR (RT-qPCR) was used to evaluate the expression of TMEM2 between L02 cells and L02 TMEM2 cells. Then, L02 cells and L02 TMEM2 cells were infected by HBV. The levels of HBsAg, HBcAg, HBV DNA and cccDNA were evaluated using immunohistochemistry and RT-qPCR.

Results: WB and RT-qPCR results showed that the expression of TMEM2 was up-regulated in L02 TMEM2 cells. Immunohistochemistry results showed that the levels of HBsAg, HBcAg were down-regulated in L02 TMEM2 cells. RT-qPCR results showed that the levels of HBV DNA and cccDNA were down-regulated in L02 TMEM2 cells.

Conclusion: TMEM2 inhibited HBV infection in L02 cells.

LBP-018

Study of the inhibitory effect of TMEM2 in HBV infection into hepatocytes

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Background: Previously, we showed that the expression of transmembrane protein 2 (TMEM2) was down-regulated in the liver tissue of patient with HBV infection and HepG2.2.15 cells. We aim to investigate the role and mechanism of TMEM2 in HBV infection in hepatocytes.

Methods: Stably knocking down (HepG2 shTMEM2) or overexpressing TMEM2 cell lines (HepG2 TMEM2 and HepG2.2.15 TMEM2) were established. The levels of HBsAg, HBcAg, HBV DNA and cccDNA were evaluated using immunohistochemistry and RT-qPCR. Western blot was used to evaluate the expression of the key components of the JAK-STAT signaling pathway. Immunofluorescence staining was employed to localize cellular interferon regulatory factor 9 (IRF9) and a luciferase reporter assay was performed to assess the influence of IRF9 nuclear translocation on interferon-stimulated response element (ISRE). RT-qPCR was used to evaluate the expression of myxovirus resistance protein 1 (MxA) and 2',5'-oligoadenylate synthetase 1 (OAS1).

Results: The levels of HBsAg, HBcAg, HBV DNA, and HBV cccDNA were up-regulated in HepG2 shTMEM2 cells while were decreased in HepG2 TMEM2 and HepG2.2.15 TMEM2 cells. The JAK-STAT signaling pathway was inhibited and activated in TMEM2-silenced cells and TMEM2-overexpressed cells, respectively. Reduced and increased expression of MxA and OAS1 were observed in TMEM2-silenced cells and TMEM2-overexpressed cells, respectively. TMEM2 did not regulate the expression of IRF9 but overexpression and knock-down of TMEM2 were found to enhance and reduce IRF9 import into the nucleus, respectively. ISRE activity was affected by alternation of IRF9 nuclear translocation.

Conclusion: TMEM2 inhibited HBV infection in hepatocytes by activating the JAK-STAT signaling pathway.

LBP-019

Urinary peptidomics for analysis of proximal renal tubular dysfunction in chronic hepatitis B

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Background and aims: Chronic hepatitis B (CHB) has been a common liver problem worldwide. Long-term treatment with nucleoside and nucleotide analogue is usually required to suppress the virus. Proximal renal tubular dysfunction (RTD) and nephrotoxicity are the infrequent but significant side effects of long-term use of nucleotide analogue. Therefore, the objective of our study was to determine the urinary peptidomic profiling of RTD in CHB patients treated with nucleotide analogue using a matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF/TOF MS).

Methods: Urine samples were obtained from 92 CHB patients with proximal RTD and 31 healthy subjects. The severity of proximal RTD can be divided into 3 groups as follow: normal (n = 68), subclinical RTD (n = 15), overt RTD (n = 9). Urine samples were purified using ZipTipC18, and then mixed with α -cyano-4-hydroxycinnamic acid matrix solution (CHCA). Urinary peptidomic profiles were acquired in a reflectron positive ion mode within a mass range of 1000–7000 Da using an Ultraflex III MALDI-TOF/TOF MS. Statistical analysis of the peptide spectra was performed with ClinProTools software.

Results: Mass spectrometry data analysis by ClinProTools revealed that there were 113 significant peptide peaks, eight of which were significantly up-regulated in severe RTD group of CHB patients as compared with those other groups ($p < 0.005$). **Conclusion:** Our results suggest that urinary peptidomic profiling analysis with MALDI-TOF MS combined with statistical methods is a rapid, accurate and reproducible approach for RTD screening. It could be used to differentiate three different stages of RTD in CHB who have been treated with nucleotide analogue.

LBP-020

Reversal of proximal renal tubular dysfunction after nucleotide analogue withdrawal in hepatitis B

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Background: Proximal renal tubular dysfunction (RTD) and nephrotoxicity are infrequent but significant complications after nucleotide analogue treatment for chronic hepatitis B (CHB). We evaluated the outcomes of proximal RTD and nephrotoxicity after nucleotide analogue withdrawal.

Methods: A follow-up study was done in CHB patients with proximal RTD after cessation of nucleotide analogue. Renal losses were defined based on the criteria of protein, glucose, phosphate, uric acid, potassium and bicarbonate. Subclinical and overt proximal RTDs were identified. The reversal and improvement of proximal RTD were evaluated at 1 year after nucleotide analogue withdrawal.

Results: From our previous study, 24/92 (26.1 %) CHB patients treated with nucleotide analogue had proximal RTD. So far, 16/17 patients have been followed up until 12 months. The median duration of nucleotide analogue taking was 70 (27–108) months. After drug withdrawal, there were overall improvement in renal function, increase in serum phosphate and uric acid, and reduction in renal losses of phosphate, uric acid and protein. The reversal of proximal RTD was seen in 13/16 (81.2 %). The patients with RTD reversal had lower renal losses of phosphate and uric acid at baseline than those in non-reversal group. Improvement of proximal RTD was seen in all, but one with overt proximal RTD. No factor was found to be associated with proximal RTD reversal and improvement.

Conclusion: Nucleotide analogue-related proximal RTD and nephrotoxicity can be reversed in most patients after drug withdrawal at 1 year. Osteopenia and osteoporosis are common problems. The outcome of abnormal bone mineralization requires further study.

LBP-021

STAT1 mediates injury of HBV-infected cell through effect of TRAIL on IFN and caspase signal pathway

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Background: Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) plays a key role in innate immune response after hepatitis B virus (HBV) infection. In our previous studies, TRAIL inhibited HBV replication through down-regulation of liver-enriched transcription factors. However, the molecular mechanism of HBV-infected cell injury induced by TRAIL remains largely unknown.

Methods: Fibrosarcoma cell lines HT1080 (STAT1+/+) and U3A (STAT1-/-) were used in this study. Rates of apoptosis in cells treated with TRAIL following transfection with HBV replication plasmid pHBV4.1 were determined by Annexin V-PI assay. Hepatitis B surface antigen (HBsAg) level in the culture supernatant were detected by enzyme-linked immunosorbent assay. The expression of interferon and caspase signal pathway proteins was evaluated by Western Blot in vitro and detected by immunohistochemistry in vivo.

Results: After addition of TRAIL, increased rates of apoptosis and reduced expression of HBsAg were observed in HT1080 cells transfected with pHBV4.1, compared with the control group and U3A cells. Expression of interferon and caspase signal pathway proteins STAT1, PSTAT1 (Tyr701), IRF7, and cleaved caspase 3 was upregulated by TRAIL in HBV-infected HT1080 cells; however, this was not detected in HBV-infected U3A cells. Expression levels of STAT1, PSTAT1 (Tyr701), IRF7, and cleaved caspase 8 were elevated in liver tissues from HBV replication mouse model after TRAIL treatment.

Conclusions: The interferon and caspase signal pathway may be involved in TRAIL-mediated cell injury via STAT1 in HBV-infected cells. Increased expression of interferon and caspase signal pathway proteins may contribute to cell injury and inhibition of HBsAg after HBV infection.

LBP-022

Analysis for 12 strains of whole sequence of HBV C/D recombinant of people in Tibet, China

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To understand the molecular genetic typing and recombinant of HBV in Tibet, the multistage random sampling was used to collect HBsAg positive samples and nested PCR was used to amplify the whole sequence of HBV. DNASTar, MEGA6 and Simplot softwares were used to assemble the sequences, make the phylogenetic tree and recombination analysis. 12 whole sequences of HBV in people of Tibetan nationality were collected by those methods. The analysis results show us that all of the 12 strains were C/D recombinants, and 9 of them the recombination spots was approximately at nt750, while the 3 of them at nt1526. Therefore those 12 strains can be divided into 2 types of recombination and named C/Da and C/Db, respectively. From the point of whole-genome sequence analysis, the 12 strains belonged to genotype C, and the nucleotide distance was more than 4 % between the 12 strains and subgenotype C1 to C15 in Genbank. They are most likely to subgenotype C1. C/Da was all collected in the middle and north Tibet such as Lasa, Linzhi and Ali, while C/Db in Shannan of south Tibet. It indicates us that the two types of recombinants distribute regularly in Tibet. The results can provide important information for the studies including special HBV recombination, gene features, virus evolution, the control and prevention policy of HBV in Tibet.

LBP-023

Increased A20 mRNA level in PBMCs is associated with immune phases of chronic hepatitis B

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The zinc finger protein A20 is a newly identified negative regulator of immune response but the role of A20 in the natural history of chronic hepatitis B (CHB) has not been demonstrated. We therefore aimed to investigate the dynamic expression of A20 and determine the potential association of A20 in the progression of chronic hepatitis B virus infection. This retrospective study contained 136 CHB patients and 30 healthy controls (HCs). The mRNA level of A20, TNF- α , NF- κ B p65 and toll-like receptor (TLR) 4 in peripheral blood mononuclear cells (PBMCs) was determined using relative quantitative real-time polymerase chain reaction. The hepatic A20 protein expression was determined by immunohistochemistry. The relative expression of A20 mRNA was significantly increased in CHB patients compared with HCs and was positively associated with alanine aminotransferase, aspartate aminotransferase, and total bilirubin. In CHB patients, the levels of A20 mRNA in the immune clearance (IC) phase and hepatitis B negative (ENH) phase were significantly higher than that in immune tolerance (IT) phase and low-replicative (LR) phase ($P < 0.001$). Furthermore, the A20 mRNA level was significantly correlated with TNF- α /NF- κ B p65/TLR4 mRNA levels in CHB patients. Of note, we reported that a cut off values of 4.19 and 3.97 for the level of A20 mRNA have significant power in discriminating IC from IT, and ENH from LR in CHB patients respectively. In conclusion, our results suggested that increased levels of A20 mRNA and protein contribute to disease progression of chronic hepatitis B virus infection.

LBP-024

The clinical significance of serum HBsAg/HBV DNA ratio in patients with chronic HBV infection

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Objective: To investigate the clinical significance of serum HBsAg-to-HBV DNA ratio in patients with chronic HBV infection.

Methods: Forty-six patients with mild to moderate chronic hepatitis B (CHB-LM), 24 patients with severe CHB (CHB-S) and 28 patients with HBV related liver cirrhosis (HBV-LC) as well as 40 CHB patients followed up 12 weeks were collected. Serum HBsAg titer and HBV DNA load were measured using the Architect QT assay and PE 9700 PCR, respectively.

Results: Serum HBsAg titer, HBV DNA load were significantly higher in CHB-LM and CHB-S groups than that in HBV-LC group, and HBsAg/HBV DNA ratio in HBV-LC group was slightly higher than that in CHB-LM and CHB-S groups. The further subgroup analysis showed that HBsAg/HBV DNA ratio were slightly increased in decompensated liver cirrhosis and non-survival patients compared with compensated liver cirrhosis and survival patients; respectively, but it was markedly elevated in HBeAg-positive group. In complete-responders and partial-responders group (12 weeks antiviral treatment), HBsAg titer and HBV DNA load were significantly decreased and accompanied with markedly increased HBsAg/HBV DNA ratio. In non-responders group, the HBV DNA load was significantly declined, and HBsAg titer was slightly declined, while HBsAg/HBV DNA ratio was slightly increased. HBsAg/HBV DNA ratio were markedly negatively correlated with PLT. The area under the curve of HBsAg/HBV DNA ratio (0.643) in predicting complete-response was higher than HBsAg (0.580) and HBV DNA (0.433).

Conclusion: The increased HBsAg/HBV DNA ratio was observed in advanced HBV-LC and HBeAg-negative patients, and it maybe related with poor therapeutic effect.

LBP-025

Hospital epidemiology of hepatitis B virus infection

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Objective: The prevalence of Hepatitis B virus infection has been greatly changed due to the extensive use of vaccination. We investigated the prevalence of hepatitis B virus infection in patients without liver diseases.

Methods: The blood samples of 231,533 patients without liver diseases who were hospitalized were detected including HBsAg, HBsAb, HBeAg, HBeAb, HBcAb, by chemiluminescence immunoassay in the first Hospital of Jilin University from October, 2014 to January, 2010.

Results: According to the history of hepatitis B vaccination in China, we divided patients into five periods, born before 1985 (period 1), between 1985 and 1991 (period 2), between 1992 and 2001 (period 3), between 2002 and 2009 (period 4), after 2010 (period 5) respectively. HBsAg positive rate was 5.73 % (10,272/179,395) in group 1, 4.33 % (726/16769) in group 2, 1.92 % (181/9431) in group 3, 0.43 % (42/9764) in group 4, 0.23 % (37/16174) in group 5. HBsAg positive rate was significantly lower in group 5 than that of other age groups ($\chi^2 = 1641.400$, $P < 0.05$). HBsAg positive rate began to decline after hepatitis B vaccination management in 1992. It further decreased due to hepatitis B vaccination in 2002. HBsAb positive rate showed it was 27.13 % (48,673/179,395), 45.63 % (7651/16,769), 57.89 % (7651/16769), 58.51 % (5713/9764), 54.69 % (8845/16174) in period 1, 2, 3, 4, 5, respectively. The increase of HBsAb positive rate shares the same reason with the decrease of HBsAg positive rate.

Conclusions: The results showed that the infection rate of hepatitis B virus in Jilin province of China was 4.86 %. This significantly decreased compared with that before hepatitis B vaccination. The main cause of the decline is the continuous increase of hepatitis B vaccinated population.

LBP-026

HBsAg decline is associated with high loss rates of HBsAg in children with HBeAg positive CHB

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Background/aim: To explore the predictive value of HBsAg during the treatment of IFN on the HBeAg/HBsAg loss in children with HBeAg positive CHB.

Methods: 200 HBeAg positive children with CHB treated with IFN alone or in combination with lamivudine for 48 weeks were enrolled. HBsAg was quantified at baseline and weeks 12, 24, 36 and 48.

Receiver operating characteristics curve was used to identify the predictive value of baseline HBsAg level on HBsAg loss, COX regression model was used to analyze the factors associated with HBeAg/HBsAg loss. Multivariable adjusted hazard ratios were derived for each factor using univariate and multivariate analysis.

Results: 82 (41 %) and 56 (28 %) patients achieved HBeAg and HBsAg loss, 67 (33.5 %) and 40 (20 %) achieved HBeAg and HBsAg seroconversion. In patients with a baseline HBsAg level <1500 IU/mL, 18 (64.3 %) achieved HBeAg loss, 14 (50 %) HBsAg loss. Compared to HBsAg <1500 IU/mL, the loss rates of HBeAg and HBsAg were lower (37.2 and 24.4 %) in baseline HBsAg <1500 IU/mL. Comparison between the patients with HBsAg <1500 IU/mL and >1500 IU/mL at weeks 12 of treatment, the former there were 41 patients achieved HBeAg loss (65.1 vs 29.6 %, $p = 0.000$), 29 HBeAg seroconversion (46.0 vs 27.2 %, $p = 0.01$), 37 HBsAg loss (58.7 vs 15.2 %, $p = 0.000$), 28 HBsAg seroconversion (44.4 vs 9.6 %, $p = 0.000$) at weeks 48 of treatment.

Conclusions: Pediatric patients with a baseline HBsAg level of >1500 IU/mL has high loss rates of HBeAg, HBsAg and HBsAg seroconversion rates. Patients with a baseline HBsAg level <1500 IU/mL but achieve a significant HBsAg decline (>1500 IU/mL) at weeks 12 of therapy is associated with high loss rate of HBeAg, HBsAg and HBeAg/HBsAg seroconversion rates.

LBP-027

Is the fetus with HBV possible infected through the route of placenta?

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Background/aim: The mechanism of intrauterine hepatitis B virus (HBV) infection remains unclear. Some reports showed that the placenta tissue is one of the transmission routes from mother to infant. HBV covalently closed circular DNA (cccDNA) is a template of HBV replication. The aim of the study is to explore the expression of HBV cccDNA in placenta tissues.

Methods: 52 pregnant women with positive hepatitis B surface antigen were enrolled. The placenta tissues were obtained from 52 patients. The samples were fixed by 10 % formaldehyde, paraffin imbedded and treated with 0.05 % poly-L-Lysine. The tissue sections were firstly treated by plasmid safe ATP dependent DNase (PSAD) so as to digest relaxed circular DNA (rcDNA) prior to RCA. Four pairs of primers were designed for mediating RCA for the first round amplification of HBV cccDNA. HBV cccDNA was further amplified by a pair of selective primers and digoxigenin labeled probes that targets the gap region between the two direct repeat regions (DR1 and DR2) of the virus after RCA. HBV DNA, HBV surface antigen (HBsAg) and HBV core antigen (HBcAg) were routinely performed.

Results: Of all patients enrolled, there was no HBV DNA, HBsAg and HBcAg positive signal was observed in the placenta tissues, in addition, there was also no HBV cccDNA positive signal was observed in the placenta tissues.

Conclusions: Placenta tissues from pregnant women with positive hepatitis B surface antigen did not support HBV replication. The transmission of HBV from mother to infant may mainly occur in the process of mother's birth.

LBP-028

Community interventions and sensitisation on hepatitis B infection with pregnant women and babies

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Background: In India, chronic HBV prevalence is 4.7 %. Vertical transmission during pregnancy and early childhood transmission accounts for nearly one-third of adult carriers. Project PAHAL, meaning “new beginning”, is an innovative partnership between United Way of Mumbai, AmeriCares India and municipal health authorities.

Methods: Training of 6409 health educators volunteers from 107 colleges to conduct awareness drives and group trainings. Targeted sensitization of 11523 reproductive age women and families (all slum residents) on prevention of HBV transmission by trained health workers. HBV education and testing of 1499 spouses and relatives of reproductive age women followed by vaccination or necessary referral. Health monitoring of 903 pregnant women—including HBV testing, health education and vaccination of infants by 6 months

Results: 430 awareness drives reaching approximately 430,000 individuals. Door-to-door sensitization of 11523 and group trainings of 2488 reproductive age women including vulnerable populations i.e. female sex workers, prison inmates. 6 of 903 pregnant women identified as HBV positive, monitored monthly until vaccination of child at 6 months. 36 of tested women, spouses and relatives identified as HBV positive, referred for management. HBV positive individuals enrolled in patient support groups. 1108 (45 %) provided with complete HBV vaccination, individuals with incomplete vaccination status scheduled for follow up.

Conclusions: The project broadened its scope to include potential sources of vertical HBV transmission—spouses and family members of child bearing women. Health educators will ensure project longevity through health camps to ensure complete HBV vaccination.

LBP-029

Pyrosequencing analysis of HBV S gene “a” determinant mutation in HBsAg⁺/Anti-HBs⁺ patients

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Background: Chronic Hepatitis B (CHB) Patients with both HBsAg and anti-HBs have been described. The mutation in the HBV S gene “a” determinant might alter the antigenic conformation and antigenicity of HBsAg. The aim of this study was to analyze the characteristics of “a” determinant mutation in CHB patients with coexistence of HBsAg and anti-HBs.

Methods: Fifty CHB inpatients and outpatients with coexistence of HBsAg and anti-HBs from our hospital were enrolled in this study. HBV S gene “a” determinant mutation was analyzed using a pyrosequencing method.

Results: HBV S gene “a” determinant mutation was observed in 17 (34 %) cases. The patterns of “a” determinant mutation (CTI-PAQGTSMFPPSCCCTKPSDGNC; aa124-147) were as follows: I126T (9, 18 %) >I126S (2, 4 %)/M133T (2, 4 %)/D144A (2, 4 %) >G130K (1, 2 %)/T131N(1, 2 %)/C137R(1, 2 %)/G145A(1, 2 %)/G145R(1, 2 %). For 9 I126T variants, T/ACU(8, 88.9 %) >T/ACA(1, 11.1 %) >T/ACC(0 %) or T/ACG(0 %). For T/ACU variants, most of T/ACU (7, 87.5 %) variants were completely mutated, while only 12.5 %(1/8) of T/ACU variants mixed with M133T isolates.

Conclusions: I126T was most frequently found in HBsAg⁺/Anti-HBs⁺ patients in our study, which was similar to other studies with genotypes B and C. We firstly showed the synonymous codon usage pattern of I126T variants in HBV “a” determinant, I/AUU to T/ACU was predominant for I126T mutation. Furthermore, we showed for the first time that C137R was found as a new substitution. This work was supported in part by the National Natural Science Foundation of China (Nos. 30800974, 81271845) and Tianjin Municipal Health Bureau of science and Technology Fund (2012KR02, 12KG118).

LBP-030

Analysis of HBV gene mutations related to NAs in chronic HBV infection

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Objective: To analyze the characteristics of HBV gene mutations related to NAs.

Methods: The data of 375 chronic HBV infected patients with resistance mutations related to NAs were analyzed.

Results: The constituent ratios of genotypic resistance accompanied biochemical breakthrough in LAM- and ADV-related resistance group were both higher than that in ETV-related resistance group. There were 16 types of mutations in 253 cases with LAM-related resistance, in which 134 cases had single-mutated sites and rtM204I was the most common mutation, 119 cases had multi-mutated sites and rtL180M + M204V was the most common mutation. rtM204I was the major locus of single-mutated sites, and rtM204V was the major locus of multi-mutated sites. Twenty-four types of mutations in 88 cases with ADV-related resistance, in which 61 cases had single-mutated sites and rtA181T was the most common mutation, 27 cases had multi-mutated sites and rtL180M + rtM204V + rtA181T was the most common mutation. Five types of mutations in 34 cases with ETV-related resistance, and all cases had multi-mutated sites, rtL180M + rtM204V + rtS202I/G was the most common mutation. The rtS202 was the major locus relative to rtT184. The mutated heterogeneities of HBV isolates were the highest in ADV-related resistance, the second in LAM-related resistance, and the lowest in ETV-related resistance. The constituent ratio of multi-mutated sites in ADV-related resistance was higher than that in LAM-related resistance.

Conclusion: The mutated sites related to NAs in HBV isolates are complex and diverse, especially in HBV isolates related to ADV resistance. We should identify with and execute preferred selection of NAs and optimal treatment. Thus it will reach the aim for preventing resistance, reducing and avoiding salvage therapy.

LBP-031

Innovative information-education-communication strategies on hepatitis B

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HBV carrier pool in India is estimated at 40 million and national prevalence rate for HBV is 4.7 %. In India, half of the CHB burden results from vertical or mother-to-child transmission, with early childhood horizontal transmission accounting for the remaining half. Efforts are needed to implement educational programs for women of childbearing age to prevent the transmission of HBV to new-borns.

Information-education-communication materials are vital to disseminate information pertaining to HBV. As part of Project PAHAL, informative collaterals were developed in common, easy to read and identify with style, in common languages, were used by College Youth trained as peer health educators through following innovative and effective strategies

Street Plays: Peer Health Educators perform street plays in busy areas, receiving attention from maximum number of people. Script of the street play is informative on HBV as well as entertaining, generating interest & attention. Then they carry placards and banners to drive the attention of the onlookers towards HBV.

Door to Door interaction: Peer Health Educators visit number of households in vulnerable communities explaining in vital details on Hepatitis. They also provide them with self-explanatory pamphlets for reference.

Focused Group Interaction: Health Educators gather number of people from the community and discuss diseases related to liver, alcoholism, pregnancy, vaccinations, etc. Such a platform becomes catalyst for the target population to understand the health issues plaguing their community in general and about HBV specifically.

LBP-032

Relationship between serum HBsAg level and intrahepatic cccDNA under different serum HBeAg statuses

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Background: This study aimed to investigate the relationships between intrahepatic cccDNA and serum HBsAg under different serum HBeAg statuses in chronic Hepatitis B patients achieved sustained virological response following antiviral therapy.

Methods: We enrolled 90 chronic hepatitis B patients with sustained virological response following antiviral therapy and divided them into 4 groups according to the serum HBeAg level. Using Real Time PCR and ELISA techniques to explore the relationships between the serum HBsAg and intrahepatic cccDNA, as well as intrahepatic HBV DNA.

Results: Intrahepatic cccDNA and HBV DNA level were higher in CHB patients than those in LC patients (both $P < 0.05$). Intrahepatic cccDNA level was positively correlated with serum HBsAg in group A (HBeAg $< 1S/CO$ ($r = 0.66$, $P = 0.02$)) and B (HBeAg 1-50S/CO) patients ($r = 0.47$, $P = 0.03$), but not in group C (HBeAg 50-100S/CO) and D (HBeAg $> 100S/CO$) patients (both $P > 0.05$). In HBeAg negative patients (group A), serum HBsAg level was correlated with intrahepatic total HBV DNA ($r = 0.52$, $P = 0.006$). However, no

relationship was found in HBeAg positive patients (group B, C and D, both $P > 0.05$).

Conclusion: Serum HBsAg can be used to predict intrahepatic cccDNA and HBVDNA level in CHB patients with low serum HBeAg status.

LBP-033

Correlation between quantitative HBsAg levels and clinical profile of chronic HBV patients

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Introduction: To determine the role of qHBsAg levels in follow up of chronic HBV(CHB)treatment and investigate the relationship between qHBsAg, HBV DNA levels and liver histopathology.

Materials and methods: 74 CHB patients visited our ID policlinic were included into the study. Patients were grouped according to treatment status; Group-I (patients new to treatment) and group-II (patients with long-term treatment history). There were 31 patients in group-I. All group-I patients had their serum HBsAg titers (qHBsAg) measured before treatment, at 3 and 6-month time periods after treatment. Group-II consisted of 43 CHB patients who had been on anti-viral treatment for at least 5 years. qHBsAg levels were measured and compared to that of patient in group II.

Results: Patients from group I were divided into two groups according to HBeAg positivity. The mean qHBsAg and HBVDNA levels and fibrosis scores were statistically higher ($p = 0.002$, $p = 0.034$, $p = 0.002$) in HBeAg positive patients. For all patients in group-I, a positive correlation was found between qHBsAg and HBV DNA levels ($p = 0.003$). Serum qHBsAg and HBVDNA levels that were measured before treatment, 3-month and 6-month after treatment were statistically different ($p < 0.05$) from each other. Group-II patients were classified according to YMDD mutations and virologic breakthrough during treatment. Serum qHBsAg levels of patients with YMDD mutations and virologic breakthrough were found to be statistically higher ($p = 0.010$) than those who did not although these patients were receiving potent antivirals.

Conclusion: Quantitative HBsAg levels may differ among chronic HBV patients according to their treatment protocols and duration. For clinicians, qHBsAg levels should not be employed only in patients undergoing interferon therapy but also those undergoing antiviral therapy as well.

LBP-034

Sensitivity of ELECSYS HBsAg II assay in detection of HBsAg mutant forms in Korean HBV patients

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Background: The immunodominant “a” determinant region (amino acids 120–147) mutations of hepatitis B virus (HBV) surface antigen (sAg) are of clinical relevance because they may affect the performance of diagnostic tests.

Methods: A total of 233 prospectively collected samples patients with chronic HBV infection from a single institution in Seoul, South Korea between January 2014 and January 2015 were included in the study. Samples were (a) characterized for major hydrophilic region and polymerase gene mutations using next generation deep sequencing to identify HBsAg mutations and (b) analyzed for the presence of HBsAg using the Roche ELECSYS HBsAg II Qualitative assay.

Results: 146/233 (62.7 %) samples had at least one of 169 different type amino-acid substitutions (distinct HBsAg mutations) in the “a” determinant HBsAg region. A total of 604 mutations were identified, located both inside ($n = 376$) and outside ($n = 228$) the HBsAg “a” determinant region. The remaining 87 (37.3 %) samples did not bear any HBsAg mutations. All 233 samples were positive for HBsAg using the ELECSYS HBsAg II Qualitative assay (sensitivity: 100 %, lower 95 % confidence limit: 98.43 %).

Conclusions: The capacity of the ELECSYS HBsAg II Qualitative assay to detect chronic HBV infection is not compromised by the known spectrum of naturally occurring HBsAg mutations in the “a” determinant HBsAg region in patients with chronic HBV from South Korea.

LBP-035

Assessment of levels of depression, anxiety and quality of life in chronic hepatitis B patients

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Background: The objective of this study was to compare the anxiety, depression and quality of life scores of chronic hepatitis B (CHB) patients and inactive hepatitis B carriers with healthy control subjects and to demonstrate the need for a multidisciplinary approach in the follow-up and treatment of patients with hepatitis B virus (HBV) infection.

Methods: The study was carried out on a total of 300 subjects; 100 CHB patients and 100 inactive hepatitis B carriers, who presented to Infectious Diseases Out-patient Clinic of Selcuk University Medical Faculty between August 2013–August 2014 and 100 healthy control subjects. All subjects were given Hospital Anxiety and Depression (HAD) Scale and Short Form-36 to assess quality of life.

Results: Based on the HAD scale, anxiety risk among the carriers was higher than the control group ($p = 0.031$). Depression risk in the patient ($p = 0.031$) and carrier groups ($p = 0.046$) were higher than

the control group. Females had higher anxiety and depression risk in the patient ($p = 0.015$ and $p = 0.037$), carrier ($p = 0.035$ and $p = 0.038$) and control ($p = 0.001$) groups. Of the Quality of life parameters, general health, role limitations due to physical health and vitality scores in the inactive hepatitis B carriers and chronic hepatitis B patients were lower than those of the controls.

Conclusions: Psychological state of the patients who are chronically infected with HBV should not be neglected during treatment and follow-up. If a psychiatric disturbance is identified, effective treatment will increase quality of life and improve compliance to treatment.

LBP-036

HBV may have an enterohepatic circulation: implications for viral persistence and tolerance

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Background: Early transmission and immunological tolerance are strong contributors to persistence of HBV. HBV has been found in neonatal gastric aspirates in association with increased risk of maternal-infant transmission. The GI tract may play a key role the pathophysiology of HBV. We investigated mechanisms by which HBV may transcytose across the intestinal mucosa to enter the circulation, and sources of potential resecretion into the gut to support an ongoing entero-hepatic circulation.

Results: Virus transcytosed across A-B polarized Caco-2 monolayers at around 3 % per hour. Exogenous human transferrin inhibited transcytosis in a dose-dependent manner, and confocal microscopy confirmed virus co-localizing with the transferrin receptor (Tfn-R) within Caco-2 cell, with strong FRET interaction between virus and Tfn-R implying co-transportation. We examined mechanisms by which virus could be chronically secreted into the intestinal lumen. Up to 12 % of HBV and duck-HBV was exported from polarized hepatocytes into the apical domain (bile). In vivo, bile from ducks infected with DHBV was found to contain high-titre virus that retained infectivity, and also viral peptides and particles that retained antigenicity. These represent a substantial antigenic load and a source of infectious virus in the gut.

Conclusion: Evidence is presented here for the release of large amount of infectious HBV, and antigenic viral peptides into the GI tract, and subsequent reabsorption across the gut epithelia by transcytosis, mediated by the transferrin receptor. These data strongly imply that HBV has an entero-hepatic circulation.

LBP-037

Metabolomic profiling of human urine from hepatitis B virus infected patients using GC/MS

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Background and aims: Chronic hepatitis B virus (HBV) infection can lead to hepatitis, cirrhosis and hepatocellular carcinoma. Hence, the development of the diagnostic tests for an early stage of chronic liver disease that are non-invasive, highly sensitive, and specific is crucial. The purpose of this study was to investigate the urine metabolomic profiles of chronic hepatitis B (CHB) in comparison to normal control subjects.

Methods: CHB patients (n = 17) and normal subjects (n = 16) were enrolled. The mean age of CHB patient and normal subjects were 46 ± 12.3 and 36 ± 10.4 years. Thirteen (23.5 %) CHB patients were male. All of them had elevated levels of serum aspartate aminotransferase (60 ± 29.1 U/L) and alanine aminotransferase (81 ± 59.4 U/L). The samples were prepared by extracting urine with tert-butyl methyl ether (TBME) and analyzed by gas chromatography/mass spectrometry (GC/MS). The acquired GC/MS data were analyzed by Mass Hunter Qualitative Analysis and Mass Profiler Professional software. Principal component analysis (PCA) was also applied to the analyzed groups to verify the distribution of the variables for the two groups.

Results: Twelve volatile organic compounds (VOCs) were shown to be significantly different between the HBV and control groups (p < 0.05). PCA model constructed by these expressed compounds showed that the GC/MS of the HBV group can be discriminated from the control group.

Conclusion: This study indicated that the GC/MS technique can be an alternative tool for the study of metabolomic biomarkers in CHB.

LBP-038

IL-9 and IL-10 but not Th9 cells are associated with survival of patients with ACLF

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CD4+ T cell are reported to be essential for the immune response to HBV infection. Th9 cells are a new subset of CD4+ T cells and function through secreting interleukin (IL)-9 and IL-10. The present study aimed to investigate Th9 cells percentage as well as IL-9 and IL-10 levels in different stages of HBV infection and their relationship with progress and prognosis of liver disease. Serum samples were collected from 26 healthy controls (HC) and 85 patients with hepatitis B virus (HBV) infection, including 39 chronic hepatitis B (CHB) patients, 25 HBV-liver cirrhosis (HBV-LC) patients and 21 acute on chronic liver failure (ACLF) patients. The Th9 cells proportion and IL-9 and IL-10 levels were determined. The results showed that no difference of Th9 cells proportion as well as IL-9 and IL-10 levels were observed in different patient groups, in patients

with different HBeAg status, and in HBV-LC patients with different complications. They were not related with inflammation index as well as prognosis indexes. In CHB patients receiving antiviral treatment, Th9 showed no change while IL-9 and IL-10 levels increased after treatment. Th9 cells showed no difference in ACLF patients survival or not, while IL-9 and IL-10 levels were significantly higher in non-survival ACLF patients. Furthermore, baseline IL-9 level showed the prognosis prediction with 87.5 % sensitivity and 61.5 % specificity for ACLF patients. Our data indicated that Th9 cells were not involved in the pathogenesis of HBV infection while elevation of IL9 and IL-10 may be related to a bad prognosis of ACLF.

LBP-039

NUCs improves the long-term prognosis in patients with CHB-associated liver failure

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Background and aims: Chronic hepatitis B (CHB)-associated liver failure (LF; CHB-LF) is associated with high mortality. Antiviral therapy with nucleoside and nucleotide analogs (NUCs) has been reported to improve the short term prognosis of CHB-LF. However, the long term effects of the therapy remain unclear. Therefore, we conducted a cohort study to investigate the long term effect of NUC-based antiviral therapy in patients with CHB-LF.

Methods: A total of 976 patients with CHB-LF were enrolled between January 2001 and December 2009 at the Liver Disease Center of Ningbo No. 2 Hospital. The patients were divided into NUCs treatment group (n = 412) and control group (n = 564). The tendency index calipers matching method was used to match the patients between the two groups to equilibrate the covariates. Survival analysis was performed using the matched samples. Cox proportional hazard model was used for the analysis the prognostic factors.

Results: (1) After the propensity matching, 262 pairs were successfully matched. No statistically significant difference was observed in the baseline characteristics of the matching pairs (p > 0.05). (2) The long term survival rate and survival duration of the NUCs treatment group were higher than that of the control group (p < 0.05). (3) Gender, age, Model for end-stage liver disease (MELD) values, cholinesterase levels, white blood cell counts, hepatic encephalopathy, concomitant infection and treatment with NUCs were found to be the independent factors associated with long term prognosis.

Conclusion: Antiviral therapy with NUCs may reduce the mortality rate and improve the long term prognosis of patients with CHB-LF.

LBP-040

HeberNasvac: rationalities and preclinical development of a novel hepatitis B therapeutic vaccine

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The therapeutic vaccine candidate (TVC) HeberNasvac, is a novel formulation based on the 1:1 combination of the recombinant HBV surface and core virus like particle antigens (HBsAg and HBcAg). HeberNasvac is produced under good manufacturing practices at the production facilities of the Center for Genetic Engineering and

Biotechnology. This vaccine candidate has been tested in different animal models in order to collect pharmacological evidences of their action mechanisms as well as the required safety preclinical data to support the product introduction in the clinical development. The present abstract summarizes the pharmacological studies in balb/c mice and transgenic mice as well as the most important toxicological studies conducted to the vaccine formulation. In general, HeberNasvac has proved to induce a strong immunological response against both HBsAg and HBeAg when administered by IN or by parenteral routes with an attractive immunomodulatory profile. In addition, acute toxicity tests, repeated toxicity studies as well as mucosal irritability study evidenced the safety of this therapeutic vaccine candidate in repeated immunization schedules combining intranasal as well as parenteral administrations. Taken together the results obtained in animal models support the introduction of HeberNasvac in the clinical practice.

LBP-041

Immunologic examinations in CHB patients after NAs treatments cessation

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Background: Virologic relapses are commonly seen in CHB patients after nucleos(t)ide analogues cessation. We aimed to find out the immunologic changes after NAs cessation.

Methods: NAs treatments were stopped in patients treated for ≥ 2 years with undetectable HBV DNA levels on ≥ 3 separate occasions 6 months apart before treatments cessation. Otherwise, HBeAg seroconversion ≥ 1 year was required in HBeAg positive patients. Virologic relapse was defined as HBV DNA >2000 IU/mL, while clinical relapse was defined as HBV DNA >2000 IU/mL and ALT $>2 \times$ ULN. Immunologic data, including CD₄⁺ T cells, CD₈⁺ T cells, CD₁₁⁺ cells, Th₁ cells, Th₂ cells, were recorded at the time of and after NAs cessation. Same examinations were recorded in healthy persons.

Results: 58 patients were recruited and 36 patients finished 48 weeks follow-up. 22 blood samples of these patients and 6 blood samples of healthy persons were examined for the immunologic index (Table 1). There were no statistical differences among healthy persons, non-relapse group and virologic relapse group.

Conclusions: No difference in immunologic index including CD₄⁺T cells, CD₈⁺T cells, CD₁₁⁺ cells, Th₁ cells and Th₂ cells could be found between non-relapse group and virologic relapse group. Advanced study should be performed to find out predictive factors for virologic relapse.

LBP-042

Efficacy of antiviral treatment for immune tolerant phase of chronic hepatitis B patients

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Background: It is uncertain that antiviral treatment for patients in immune tolerant phase leads to better prognosis. The aim of this study is to assess the impact of antiviral treatment for immune tolerant chronic hepatitis B (CHB) patients.

Methods: This study included 595 patients diagnosed as immune tolerant phase of CHB without LC in two large volume medical centers in Korea between 2006 and 2010. The patients were categorized in two groups: antiviral treatment group (n = 60) and control group (n = 535). The primary endpoint was overall survival (OS) and secondary endpoints included development of HCC and LC. To compare the endpoints, Kaplan–Meier method and Cox regression analysis were used. Inverse probability weighting (IPW) was used to balance the baseline characteristics of the two groups (Fig. 1).

Results: In multivariate Cox analysis, the treatment group showed significantly lower risk of HCC than the control group (hazard ratio [HR] = 0.196; 95 % confidence interval [CI] 0.054–0.710; P = 0.013). Antiviral treatment decreased the risk of LC compared to the control group (HR = 0.399; 95 % CI 0.184–0.864; P = 0.019) as well. The treatment group showed longer OS than the control group (HR = 0.140, 95 % CI 0.019–1.028; P = 0.053), although it failed to reach statistical significance. When the two groups were balanced by IPW, the risk of HCC (HR = 0.083; 95 % CI 0.028–0.250; P < 0.001) and LC (HR = 0.333, 95 % CI 0.117–0.949; P = 0.039) were significantly lower in treatment group. Also antiviral treatment prolonged OS significantly compared to the control group (HR = 0.106, 95 % CI 0.024–0.472; P = 0.003).

Conclusion: Antiviral therapy even for immune tolerant patients may prolong OS and reduce the risk of HCC and LC.

Table 1 Immunologic Index in healthy persons, non-relapse and virologic relapse

	Healthy persons n=6	Non-relapse n=20	Virologic relapse n=2	F	p
CD ₄ ⁺ T(%)	45.4 ± 10.0	46.1 ± 15.2	46.9 ± 1.9	0.01	0.990
CD ₈ ⁺ T(%)	45.7 ± 10.7	38.7 ± 9.8	45.4 ± 2.5	1.444	0.255
CD ₄ ⁺ T/CD ₈ ⁺ T	1.06 ± 0.37	1.39 ± 0.92	1.04 ± 0.11	0.478	0.625
CD ₁₁ ⁺ C(%)	20.9 ± 16.4	22.2 ± 15.2	23.3 ± 15.3	0.024	0.977
Th1(%)	6.5 ± 6.5	8.6 ± 7.1	5.0 ± 3.0	0.426	0.658
Th2(%)	8.3 ± 1.1	7.5 ± 6.5	9.8 ± 7.7	0.147	0.864
Th1/Th2	0.74 ± 0.73	1.63 ± 1.37	0.57 ± 0.14	1.622	0.218

LBP-043

AFP level is a useful predictor of HCC in chronic hepatitis B patients treated with NUCs

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Objectives: To evaluate the predictive factors for the occurrence of HCC during the NUC treatment in patients with chronic hepatitis B (CHB).

Methods: CHB patients treated with NUCs for at least 2 years were studied. The association between HCC occurrence and several factors such as gender, age, serum ALT and AFP levels, HBeAg status, serum HBV-DNA level, and presence or absence of cirrhosis before

and 1 year after the commencement of NUC treatment, were retrospectively analyzed.

Results: Total 139 patients (male, 64.7 %; mean age, 50.2 ± 12.0 years; cirrhosis, 33.9 %) were included. Mean follow-up periods were 80.1 ± 40.7 months. Sixteen-six (47.5 %) and 73 (52.5 %) were initially treated with lamivudine or entecavir, respectively. During the follow-up periods, HCC developed in 13 (9.4 %) of total patients. Mean duration between the NUC start and HCC occurrence were 59.0 ± 32.8 months. Univariate analysis showed that older age (>50 years), baseline serum ALT level >30 IU/mL, serum AFP level >10 ng/mL at year 1, and the presence of cirrhosis, were significantly correlated with the HCC occurrence. Multivariate analysis showed the older age, high AFP level at year 1, and presence of cirrhosis were the independent predictor of HCC occurrence. Kaplan–Meier analysis showed patients with older age, cirrhosis, or serum AFP level >10 ng/mL at year 1 developed HCC more frequently than patients without these risk factors.

Conclusion: Serum AFP level at year 1 after the commencement of NUC treatment could be a useful marker of HCC occurrence for HBV patients treated with NUCs.

LBP-044

A novel regulatory mechanism of hepatic myeloid-derived suppressor cell accumulation

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Background: Myeloid-derived suppressor cells (MDSCs) have been shown to accumulate in the liver and thus promote tumor growth. The potential mechanism involved in hepatic MDSCs accumulation is still under investigation.

Methods: We prepared liver fibroblast stromal cells to mimic the hepatic microenvironment. The frequency and the phenotype of MDSCs were analyzed by flow cytometry. Tumor-induced MDSCs were isolated and purified from bone marrow for functional analysis. MDSCs migration was evaluated using an in vitro chemotactic assay and an in vivo adoptive cell transfer assay. In some experiments, specific neutralizing antibodies were used.

Results: The frequency of MDSCs increased in the bone marrow, spleen, blood and liver in tumor-bearing mice. Intravenously injected MDSCs primarily accumulated in the liver. Liver fibroblast stromal cells secreted higher levels of cytokines and chemokines, including G-CSF, GM-CSF, IL-6, MCP-1 and SDF-1, after treatment with tumor-conditioned supernatant. Further analysis indicated a continuously increased level of cytokine expression in liver tissue during tumor growth. Tumor-activated liver stromal cells promoted MDSCs migration and accumulation into the liver and tumor site, and the blockade of MCP-1 and SDF-1 using neutralizing antibodies reduced MDSCs infiltration at the tumor site and decreased tumor growth.

Conclusion: These findings demonstrate a novel regulatory mechanism of hepatic MDSCs accumulation mediated by liver stromal cells. Tumor-activated liver stromal cells produce higher levels of chemokines and cytokines, which contribute to MDSCs accumulation in the liver.

LBP-045

Up-regulation of tumoral IDO is driven by monocytes and T lymphocytes collaboration

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Indoleamine 2, 3-dioxygenase (IDO) in cancer cells plays a critical role in tumor immunosuppression. However, the precise mechanisms regulating IDO expression in tumor cells remain unclear. Here, we reported that IDO expression in tumor cells of various human cancer types, including hepatocellular, cervical and lung carcinomas, displayed a discrete rather than uniform pattern. 9 human cell lines of these cancer types did not constitutively express IDO in vitro. Interestingly, co-culture with peripheral blood mononuclear cells (PBMC) significantly induced and maintained IDO expression in these tumor cells, predominantly through IFN- γ . Mechanistically, we showed that IDO expression in tumor cells was only induced when co-cultured with both T lymphocytes and monocytes. Moreover, the cooperation between T lymphocytes and monocytes played an indispensable role on the tumoral IDO expression in immunocompromised mice. Taken together, our data supported the notion that IDO expression in tumor cells might serve as a counter-regulatory mechanism regulated by immune response, rather than a tumor-intrinsic event, and provided new insights into the collaborative action of different inflammatory cells in tumor immunosuppression.

LBP-046

Proteomic analysis for Identification of biomarkers in non-B, non-C hepatocellular carcinoma

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Aims: We previously reported proteomic biomarkers of hepatitis B virus (HBV) related Hepatocellular carcinoma (HCC) including moesin, methionine adenosyl transferase, human liver carboxylesterase and acyl-CoA dehydrogenase. In this study, we used a proteomic approach to identify the differentially expressed protein biomarkers of HCC developed from non-B, non-C (NBNC) hepatitis.

Methods: 4 cases of NBNC HCC patients had undergone partial hepatectomy for HCC and, the tissue samples were analyzed by 2-DE combined with matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF-MS). The differential expression in protein profiles of HCC were compared to those of adjacent non-tumorous liver tissues.

Results: 37 protein spots were found significantly changed in HCC compared with adjacent non-tumorous liver tissues. 17 proteins were up-regulated, whereas the other 20 proteins were down-regulated in tumor tissues. 7 proteins including heat shock protein, cytokeratin 9, pyruvate kinase, nucleophosmin, aldose reductase, peroxiredoxin-1 and NADH dehydrogenase 1 were identified from up-regulated spots in HCC. 6 proteins including carbamoyl-phosphate synthase, human liver carboxylesterase 1, betaine-homocysteine methyltransferase, acetoacetyl-CoA thiolase, aflatoxin B1 aldehyde reductase and 4-hydroxyphenylpyruvate dioxygenase were identified from down-

regulated spots in HCC. 3 proteins identified in NBNC HCC were identical with those found in HBV related HCC. And these proteins are associated with oxidoreduction, methionine and fatty acid metabolism.

Conclusions: Proteomic study using MALDI-TOF MS is an efficient strategy for identifying differentially expressed proteins in HCC. Identification of potential biomarkers in HCC by proteomic approach may provide further insights into pathogenesis of NBNC HCC as well as steatohepatitis related HCC.

LBP-047

M-CSF receptor antagonist inhibits progression of hepatocellular carcinoma in vivo

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Aim: The purpose of this study was to investigate effects of M-CSF receptor antagonist on HCC.

Materials and method: (1) Isolated mouse hepatic macrophages (Mfs) or monocytes (Mo) were cultured with media added the different dose of M-CSF. Production of vascular endothelial growth factor (VEGF) by isolated macrophages Mfs or Mo was assessed. Furthermore, isolated vascular endothelial cells (VEC) were co-cultured with or without hepatic Mfs in presence with M-CSF and cell proliferations were assessed in vitro. (2) Mice were treated with diethyl nitrosamine (DEN) intraperitoneally. For treatment group, M-CSF receptor antagonist (GW2580) was treated. Incidence of tumors, angiogenesis and distribution of M2Mfs were assessed. (3) Mouse HCC cells were implanted to same mice strain by subcutaneous injection. Tumor progression was assessed. (4) Human HCC cells (Huh7) were implanted into the spleen in the nude mice injection. Tumor progression was assessed.

Result: (1) The production of VEGF was significantly greater in hepatic Mfs compared with that in Mos. Proliferation of VEC was greatest in cells cultured with hepatic Mfs in media with M-CSF. (2) Hepatic tumors were observed in animals treated with DEN. In contrast, tumor incidence was significantly reduced in animals with GW2580. Enhanced angiogenesis M2Mf population observed in the DEN-treated animals was significantly blunted by treatment of GW2580. (3) and (4) Growth of implanted both of HCC was significantly inhibited by GW2580 in vivo.

Conclusions: M-CSF and/or its receptor could be a new therapeutic target for HCC.

LBP-048

Targeted and exome sequencing analysis of hepatocellular carcinoma

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Aim: Genetic analysis has revealed a subset of recurrently mutated genes and aberrant cellular signaling pathways in hepatocellular

carcinoma. To reveal the genetic alterations and dysregulated pathways in hepatocellular carcinoma, we performed targeted sequencing and exome analysis using next-generation sequencer.

Methods: We analyzed the somatic mutational profiles of 16 genes in primary hepatocellular carcinoma by targeted ultra deep sequencing using nine pairs of specimens (tumor and peripheral blood) and whole-exome sequencing using one pair of samples.

Results: Overall, somatic mutations with high allele fraction were identified in tumor tissues by targeted deep sequencing. Somatic mutations with high allele fraction were observed in TP53 (3/9; 33 %) and CTNNA1 (2/9; 22 %) genes in five out of nine (55 %) specimens. In vitro analysis showed CTNNA1 H36P mutant protein identified in tumor samples was resistant to protein degradation and promoted cell proliferation. Exome sequencing identified SLIT3 mutation, implying the dysregulation of axon guidance genes involved in the development of hepatocellular carcinoma. These results showed TP53 and WNT/beta-catenin signaling pathways were commonly mutated in hepatocellular carcinoma.

Conclusions: These results suggested targeted sequencing and exome sequencing enable us to identify putative oncogenic driver mutations during the development of hepatocarcinoma.

LBP-049

Somatic mutations in non-tumorous fibrotic tissue

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Aim: Virus infection or metabolic stress causes liver damage including chronic hepatitis and cirrhosis with deposited connective tissue (fibrosis), which indicates premalignant conditions for HCC. However, the mutational status in premalignant conditions such as chronic hepatitis and cirrhosis remains unknown.

Methods: We analyzed the somatic mutational profiles of 16 genes in peripheral blood, tumor and non-tumorous tissues by targeted sequencing using nine pairs of specimens.

Results: In four out of nine patients, a variety of somatic mutations with low allele fraction were detected by targeted deep sequence (average coverage depth 3549-folds). Furthermore, the number of mutations was significantly similar in matched tumors and non-tumor tissues ($R^2 = 0.89$), suggesting that background noise was similar in tumor and non-tumor tissue (Fig. 2B). The fibrotic stage correlated with the accumulation of mutations in non-tumorous tissues.

Conclusions: These results suggested that somatic mutations accumulated even in the premalignant condition, and subsequently oncogenic driver mutations occur and promote hepatocarcinogenesis.

LBP-050

Effects of androgen receptor on fatty acid metabolism in human hepatoma cell lines

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Background and aims: Hepatocellular carcinoma (HCC) is one of the male-dominant diseases and androgen receptor (AR) seems to play an important role in hepatocarcinogenesis. Fatty acid metabolism also plays a role in human carcinogenesis and is associated with the prognosis of cancers. This study aimed to explore the effects of AR on fatty acid metabolism-associated gene expression in human hepatoma cell lines.

Methods: AR-expression plasmid or control plasmid was transiently transfected into human hepatoma cell lines Huh7 or HepG2. After 48 h, cellular protein and RNAs were extracted. Expression of AR was confirmed by western blotting using specific antibodies. cDNA was synthesized and subjected to real-time PCR-based array to examine expression of 84 fatty acid metabolism-associated genes.

Results: In Huh7 cells, AR significantly down-regulated expression of 11 fatty acid metabolism-associated genes: 4 acyl-CoA synthetases (ACSM2A, ACSL6, ACSL3, and ACSM3), 3 acyl-CoA thioesterases (ACOT7, ACOT9, and ACOT6), 1 acyl-CoA oxidase (ACOX3), 1-fatty acid transport (SLC27A2), 1 fatty acid biosynthesis regulator (PRKAG2) and 1 ketogenesis and ketone body metabolism (BDH2). In HepG2 cells, AR significantly up-regulated expression of 6 fatty acid metabolism-associated genes (PRKAG2, PPA1, ACOT9, SLC27A5, SLC27A2, and FABP4) and significantly up-regulated expression of 35 fatty acid metabolism-associated genes. ACOT7 and ACOX3 were down-regulated in both cells.

Conclusion: AR leads to the disturbance of fatty acid metabolism-associated gene expression in human hepatocytes. Modulation of cellular acyl-CoAs by AR could play a role in hepatocarcinogenesis through the regulation of various fatty acid metabolism

LBP-051

SNPs analysis of oligo-adenylate synthetase and Interleukin 1B genes in end-stage liver disease

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SNPs are single-base inheritable variations in a given and defined genetic location that occur in at least 1 % of the population. Analysis of DNA sequences shows considerable variability between individuals. The opportunity to rapidly detect unique SNPs and other DNA point mutations offers potential for developing personalized medicines with an advantage of early HCC diagnosis and treatment.

Aim of the work: This study was designed to assess the SNP analysis of IL1B and OAS in patients with HCV related end stage liver diseases (liver cirrhosis and HCC) to assess the ability to be used for early prediction of HCC.

Methods: Whole blood samples were obtained from 25 HCC patients, 25 HCV-related liver cirrhosis in addition to 25 HBV and HCV seronegative controls. Routine clinical, laboratory and ultrasonographic assessments were done. HCC was diagnosed according to AASLD guidelines. SNP analysis was assessed by DNA specific restriction enzymes polymorphism.

Results: SNP analysis of IL1B and OAS genes was detected with different frequencies in the HCV related CLD including HCC and also in control group with significant difference between the studied groups.

Conclusion: SNP analysis of IL-1 B and OAS can be used as molecular markers for early diagnosis of hepatocellular carcinoma in HCV related chronic liver diseases.

LBP-052

The meaning of AFP, AFP-L3 and PIVKA-II for evaluating recurrence in patients with HCC

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Backgrounds: Alpha-fetoprotein (AFP) and prothrombin time induced by vitamin K absence-II (PIVKA-II) have been used a diagnostic and surveillance marker for hepatocellular carcinoma (HCC). However, many patients showed less level than optimal level that previously reported in many studies. We investigated the meaning of the AFP and PIVKA-II, additionally lens culinaris agglutinin-reactive fraction of AFP (AFP-L3) for evaluating recurrence of HCC.

Methods: We retrospectively reviewed the medical records of patients who underwent liver resection. We investigated the recurrence and association with AFP, AFP-L3 and PIVKA-II levels. Results Median AFP, AFP-L3 and PIVKA-II were 8.5 ng/dL, <0.5 % and 24 mAU/mL, respectively. Median tumor size was 2.4 cm. Six patients (8.6 %) had grossly portal vein invasion (PVI). Microscopic PVI was found in 8 patients (11.4 %). During median 17 month follow-up period, 19 patients had recurrence. A univariate analysis revealed that delta AFP-L3, tumor size, gross PVI, satellite nodule, microscopic PVI, bile duct invasion and microvessel invasion were significant prognostic factors of recurrence. However, multivariate analysis revealed that only delta AFP-L3 less than 50 % was significant prognostic factors.

Conclusions: As the surveillance program has been generalized in patients with high risk of HCC, identification of early and resectable HCC has been increased. However, many patients showed normal level of tumor marker and surgical resection showed still high recurrence rate. Although more large-scaled study will be needed, we may predict the recurrence risk according to the change of AFP-L3.

LBP-053

Etiology of chronic liver disease and HCC biomarkers

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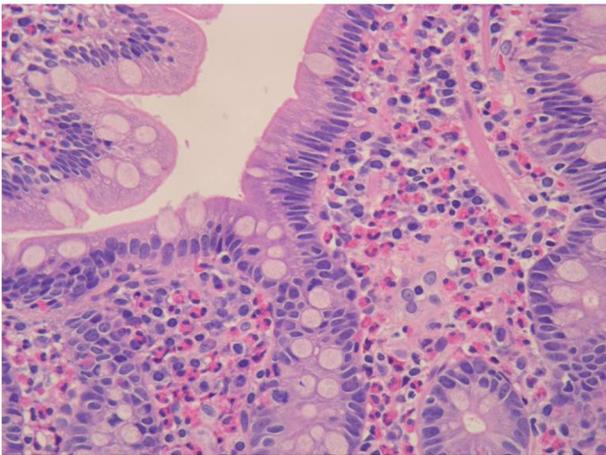
Background and aim: The role of serum biomarkers in HCC diagnosis is controversial. We studied the diagnostic performances of α -fetoprotein (AFP) and prothrombin induced by vitamin-K absence-II (PIVKA-II) in HCC and cirrhotic patients of different etiology.

Patients and methods: Biomarkers levels were quantified by chemoluminescent-enzyme-immunoassays on Lumipulse-G-System (Fujirebio Inc, Tokyo, Japan) at the time of diagnosis in 258 HCC patients (204 males; mean-age, 65.5 ± 10.1 years) and at a single point during follow-up in 130 Cirrhotics non-HCC (104 males; mean-age, 62.8 ± 9.6 years). Patients (HCC/non-HCC) were recruited consecutively at 3 Hepatology Centres and classified as Chronic Hepatitis-B (48/35), Chronic Hepatitis-C (126/56) and Non-Viral (84/39).

Results: Both AFP and PIVKA-II values were significantly higher in HCC than cirrhotics ($p < 0.001$), but correlated with low ρ in each group (HCC: $\rho = 0.281/p < 0.001$; cirrhotics: $\rho = 0.255/p = 0.006$). AFP positively correlated with ALT in HBV cirrhotics ($\rho = 0.626/p < 0.001$), on nucleos(t)ide analogues, but not in the other cirrhotics. PIVKA-II poorly correlated with ALT (HCC: $\rho = 0.176/p = 0.068$; cirrhotics: $\rho = 0.282/p = 0.030$). AFP correlated with tumorsize in HBV-patients only ($\rho = 0.414/p = 0.008$) whereas PIVKA-II was positively correlated in all groups (HBV: $\rho = 0.545/p < 0.001$; HCV: $\rho = 0.422/p < 0.001$; Non-viral: $\rho = 0.538/p < 0.001$). AUROC curve was slightly higher for PIVKA-II than AFP overall (0.764/0.647) and in each etiology group (HBV: 0.833/0.822; HCV: 0.732/0.648; Non-Viral: 0.806/0.640). Diagnostic performance at multiple cut-offs showed different results according to etiology (table) and the AFP + PIVKA-II-combination increased sensitivity, specificity and diagnostic accuracy of HCC diagnosis in Non-viral and HCV better than in HBV etiology (Figs. 1, 2).

Conclusion: The diagnostic performances of AFP and PIVKA-II in HCC and Cirrhotics patients differed according to liver disease etiology. Our findings foster prospective studies to identify specific etiology-based cut-off to warrant a personalized use of HCC biomarkers.

Picture 1 Pathologic examination shows marked eosinophilic infiltration in the intestinal mucosa



Picture 2 The abdominal computed tomography demonstrated moderate ascites and diffuse wall thickening involving the duodenum, jejunum, terminal ileum and ascending colon.

LBP-054

Des- γ -carboxy prothrombin is a complementary biomarker in diagnosing Hepatocellular Carcinoma

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The incidence of hepatocellular carcinoma (HCC) is increasing in China. AFP had been used in diagnosing HCC for a long time with unsatisfactory sensitive and specificity. Des- γ -carboxy- prothrombin (DCP) was a potential surveillance tests for HCC. This study was to evaluate the diagnosing value of AFP combined DCP for HCC. We performed a single-center study. We determined serum concentrations of AFP and DCP of 625 patients with liver diseases. AFP and DCP were elevated in 625 patients. One-third of them were cirrhotic patients due to hepatitis B or C viruses (HBV/HCV). Forty percent of patients were chronic HBV/HCV infection. There were 77 HCC patients. All the HCC patients showed a much higher DCP when compared with non-HCC patients, $P < 0.04$. Fifteen patients with CT/MRI identified HCC had DCP < 40 mAU/ml and AFP > 10 ng/ml. Among 15 patients with image confirmed HCC had higher DCP and AFP level. We found 27 patients with image or surgeon confirmed HCC showing abnormal DCP and normal AFP. By combined AFP at cutoff 10 ng/ml and DCP cutoff 40 mAU/ml, the specificity increased to 90.2 % and the sensitivity was 85 %. The area under the curve for AFP combined DCP was 0.90). We found DCP > 500 mAU/ml indicated metastasis or relapsing of HCC. The are under the curve for DCP in predicting metastasis or relapsing of HCC was 0.69. Our results indicted DCP was a complementary biomarker in diagnosing HCC. DCP higher than 500 mAU/ml warned metastasis or relapsing of HCC.

LBP-055

Characteristics of HCV-related HCC in Pakistan**Javed I. Farooqi¹, Rukhsana J. Farooqi², Qasim Jan¹**¹Department of Medicine, Lady Reading Hospital, Khyber Medical University, Peshawar, Pakistan; ²Khyber Teaching Hospital, Peshawar, Pakistan

Hepatocellular carcinoma is the fatal complication of liver cirrhosis. In Pakistan, HCV-related cirrhosis is the most common among cirrhotic population. We report different characteristics of HCC in HCV-related cirrhosis.

LBP-056

Aflatoxin role in liver cancer in Nile delta**Sherief M. Abd-El salam¹, Mohamed A. Sharaf-Eldin¹, Raafat A. Salah¹, Hanan H. Soliman¹, Said H. Abdou², Walaa A. El Khalawany¹**¹Tropical Medicine Department, Tanta University, Tanta, Egypt;²Clinical Pathology Department Tanta University, Egypt

Background and aim: The burden of hepatocellular carcinoma (HCC) has been increasing in Egypt. This fact raises the question, does the high prevalence of HCC in Egypt is related only to HCV? or is it augmented by AFB1 exposure in our patients? The aim of this study was to identify the role of aflatoxin as an environmental risk factor attributable to liver cancer in Nile delta.

Methods: This cross sectional study was carried out in tropical medicine department in Tanta university hospital on 40 patients with HCC, 20 cirrhotic patients. Fifteen individuals were invited to share in the study as a control group. All patients and control were evaluated for age, sex, residence, occupation, viral markers, liver functions and serum level of aflatoxinB1.

Results: Aflatoxin level in serum was significantly higher in HCC patients when compared to cirrhotics and to controls. The mean age of HCC patients was 58.575 + 9.583 years. HCC was much higher in males than females with male to female ratio 4.7:1. Concerning smoking, 45 % of HCC patients were smokers. DM was diagnosed in 42.5 % of HCC cases. Anti HCV-Ab was present in 95 % of HCC cases.

Conclusions: Environmental aflatoxin seems to be a major risk factor for HCC in Nile delta. There is more than synergy between HCV and Aflatoxin as chronic hepatitis could render the liver less capable of removal of Aflatoxin from the body. Also, Aflatoxin may induce mutation in p53 paving the way for HCV to induce HCC.

LBP-057

Pre-clinical studies of animal irreversible electroporation treatment of liver cancer**Jing Xie, Zhou_yu Ning, Zhi_qiang Meng**

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Objective: To investigate the apoptosis, antitumor effect and security of IRE technique for liver cancer xenograft.

Methods: 12 New Zealand white rabbits with liver cancer xenograft models were randomly divided into control group and treatment group. Rabbits were executed after IRE. Liver ? kidney function and creatine kinase were tested, Elisa methods were used to detect CTnI, Caspase-3, TNF- α and VEGF factor levels in the plasma. Tumor was fixed with POM, embedded, sectioned, and HE stained. TUNEL assay was used to detect the apoptosis. Immunohistochemistry was applied to detect Bcl-2, HSP70 and VEGF expression in liver cancer xenograft.

Results: Elisa of plasma showed IRE effectively increase the body Caspase-3, TNF- α secretion, while reducing VEGF expression, with no elevation of CTnI. HE staining showed a clear borderline between the necrotic and normal area. A large number of red blood cells and inflammatory cell infiltration on the edge. After 14 days of the treatment, regeneration signs appeared in the target tumor tissue. TUNEL assay showed that IRE can significantly increase the apoptosis in liver cancer xenograft cells compared with the control group. Immunohistochemistry results showed that IRE can decrease the expression of Bcl-2 and VEGF, as well as increase HSP70 expression in liver cancer xenograft, and improve anti-tumor effect of IRE.

Conclusions: IRE can inhibit the growth of liver tumors via inhibiting angiogenesis, inducing apoptosis and producing specific anti-tumor immune response. IRE is a safe and effective approach for liver cancer.

LBP-058

Late-onset diaphragmatic hernia after radiofrequency ablation of hepatocellular carcinoma**Hitomi Takechi, Tomoyuki Abe, Ryusuke Saito, Nobuaki Fujikuni, Tatsunari Sasada, Makoto Yoshida, Minoru Yamaki, Hironobu Amano, Masahiro Nakahara, Toshio Noriyuki**

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Background: Percutaneous radiofrequency ablation (RFA) is widely used as an effective treatment for liver tumors. Several reported complications associated with RFA are due to thermal damage of neighboring organs. The present case reports diaphragmatic hernia in association with RFA and hepatocellular carcinoma (HCC).

Case: A 72-year-old woman with HCCs of S5 and S8 was treated repeatedly by RFA and transcatheter arterial chemoembolization for 3 years. After the third round of RFA to target S5 HCC recurrence, acute abdominal pain and dyspnea suddenly occurred. The vital signs were not remarkable, but acute pain was found in the right hypochondrium. Laboratory findings did not demonstrate any abnormalities, and a contrast-enhanced computed tomography revealed intrusion of the transverse colon through the right diaphragm hernia. Additionally, the colon was dilated and showed changes suggestive of ischemic conditions. An emergency surgery was performed to close the hernia by non-absorbable strings to preserve the colon. The intruded transverse colon did not show necrosis. This patient was discharged without any complications 13 days following the surgery.

Consideration: The first-line treatment for this disease involves surgical intervention. Diaphragm hernia is a very rare complication of RFA. The present case suggests that patients who underwent several rounds of RFA require surveillance of diaphragmatic hernias. Special attention to diagnose the delayed complications of RFA, such as diaphragmatic hernia, is warranted in such cases.

LBP-059

Clinical characteristics and treatment outcomes of HCC due to CHB and CHC infections in Thailand**Ratha-korn Vilaichone, Nattawat Wanich, Krittapong Somboon, Sith Siramolpiwat**

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Background: Hepatocellular carcinoma (HCC) is the important cancer related death in Asia–Pacific region. This retrospective study was designed to assess clinical characteristics, treatment outcomes and prognosis of HCC due to chronic hepatitis B (CHB) and chronic hepatitis C (CHC) infection in Thailand.

Methods: This retrospectively study was enrolled HCC patients from CHB and CHC infections aged more than 15 years old in Thammasat university hospital, Pathumthani, Thailand from January 2007 to December 2014. Clinical information, treatment outcomes and prognosis were collected from review of medical records.

Results: There were 90 cases with HCC including 70 patients from CHB infection and 20 patients from CHC infection. The common presentations were hepatomegaly 50/90 (55.6 %), abdominal pain 38/90 (42.2 %) and ascites 18/90 (20 %). Cirrhosis was detected in most patients (75/90 (83.3 %)). Based on Barcelona Clinic Liver Cancer (BCLC) staging, 68/90 (75.6 %) presented with intermediate or late stage. Patients received curative therapy (surgery or radiofrequency ablation) had significantly longer survival time than palliative therapy group (12 vs 3 months, p -value <0.05). Patients with advanced stage had poorer prognosis than early stage. The mean survival time after HCC diagnosis was 12 months.

Conclusion: CHB and CHC infections remain the major causes of HCC in Thailand. Patients presented with early stage and received curative therapy had longer survival time than palliative treatment. Most patients with advanced diseases had poor prognosis. High suspicious and appropriate HCC screening program for CHB and CHC patients might be important tools to early detection and improve survival rate in these high risk patients.

LBP-060

Oral medications improve overall survival by enhancing compliance to surveillance for HCC**Joon Yeul Nam¹, Jeong-Hoon Lee¹, Jieun E Kim², Dong Hyeon Lee¹, Young Chang¹, Hongkeun Ahn¹, Hyeki Cho¹, Jung-Ju Yoo¹, Minjong Lee¹, Young Youn Cho¹, EunJu Cho¹, Su Jong Yu¹, Yoon Jun Kim¹, Jung-Hwan Yoon¹**

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Background/aims: The regular surveillance for hepatocellular carcinoma(HCC) in chronic hepatitis B(CHB) patients is essential to detect HCC earlier and to improve their prognosis. This study aimed to determine whether the prescription of oral medications which might increase the perceived severity according to the Health Belief Model, contributes to early tumor detection and overall survival.

Methods: A total of 401 CHB patients who were newly diagnosed as HCC were included: 134 patients received no medications (group1), 151 received hepatotonics (group2), and 116 received nucleos(t)ide analogues(NAs) (group3) within 2 years before HCC diagnosis. Primary endpoint was overall survival and secondary endpoints were compliance to surveillance and initial HCC status.

Results: The rates of good compliance to surveillance (defined as >80 % of visit intervals were <6 months) was higher in both group 2 (95.0 %) and 3 (96.7 %) than in group 1 (63.4 %) (all $P < 0.001$). HCC diagnosed in early stage (BCLC 0) was higher in both group 2 (32.5 %) and 3 (36.2 %) than in group1 (20.9 %) (all $P < 0.05$). HCC diagnosed in advanced or end stage (BCLC C, D) was significantly lower in both group 2 (37.1 %) and 3 (32.8 %) than in group 1 (42.5 %) (all $P < 0.05$). Mean maximal tumor size was significantly smaller in both group 2 (1.9 ± 1.1 cm) and 3 (1.8 ± 0.9 cm) than in group 1 (2.8 ± 2.4 cm) ($P < 0.001$). Comparing to group 1, group 2 (HR, 0.63; 95 % CI 0.41–0.97; $P = 0.032$) as well as group 3 (HR, 0.396; 95 % CI 0.221–0.711; $P < 0.001$) showed significantly longer overall survival.

Conclusions: The prescription of medication improves the patients' compliance to surveillance and enables the early detection of HCC which is associated with survival gain.

LBP-061

Liver function assessment according to the ALBI grade in sorafenib-treated advanced HCC patients**Sadahisa Ogasawara, Tetsuhiro Chiba, Yoshihiko Ooka, Eiichiro Suzuki, Naoya Kanogawa, Tomoko Saito, Tenyu Motoyama, Akinobu Tawada, Osamu Yokosuka**

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Background: The Albumin–Bilirubin (ALBI) grade has been proposed as a new, simple, and objective method of assessing liver function. Further data are required to validate the utility of the ALBI grade as a measure of liver function of sorafenib–treated patients with advanced hepatocellular carcinoma in clinical practice or research trials.

Methods: We evaluated the correlations between the ALBI grade and Child–Pugh score, adverse events, and survival in 89 patients with advanced HCC who were prospectively treated with sorafenib.

Results: Majority of patients with ALBI grade 1 (14/15 patients, 93 %) had a Child–Pugh score of 5. Patients with ALBI grade 2 had a wide range of liver function according to the Child–Pugh scores, with scores of 5, 6, 7, and ≥ 8 . We subdivided patients with ALBI grade 2 into two groups according to the ALBI score (grade 2A, ≤ -2.118 and grade 2B, > -2.118). Overall survival in the ALBI grade 2B group was significantly shorter than that in the ALBI grade 1 and 2A groups. Thus, ALBI grade 2B was an independent predictor of poor prognosis in addition to elevated serum aspartate aminotransferase levels, increased serum alpha-fetoprotein level, and macrovascular invasion.

Conclusion: Sorafenib may be indicated for patients with ALBI grade 1 and a subset of patients with ALBI grade 2. We consider the division of patients with ALBI grade 2 into subgroups based on continuous variables as a reasonable approach to further identify patients indicated for sorafenib therapy.

*LBP-062***Efficacy of sorafenib in patients with intermediate-stage HCC refractory to TACE**

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Background and purpose: Induction of sorafenib is recommended for patients with intermediate-stage hepatocellular carcinoma (HCC) refractory to transcatheter arterial chemoembolization (TACE). The aim of this study is to evaluate the efficacy of sorafenib in such patients, in comparison with continued TACE therapy.

Method: This retrospective cohort study reviewed 17 patients who developed intermediate-stage HCC after TACE treated at Jikei University Hospital between June 2009 and December 2015. The patients with TACE-refractory intermediate-stage HCC were divided into two cohorts: (1) those who switched from TACE to sorafenib ($n = 10$) and (2) those who continued TACE ($n = 7$). We evaluated the patients' background, clinical data, overall survival (OS) and the time to disease progression (TTDP: the time patients developed advanced-stage HCC) after they were judged refractory to TACE.

Results: When the patients were judged refractory to TACE, no significant differences were present in PT and serum T-Bil as well as Alb between the two groups. The median TTDP and OS were 444.5 and 604.5 days for sorafenib group, and 87.5 and 36 days for TACE group, respectively ($p = 0.0894$ and 0.5582 , respectively). Median serum albumin level at 3 and 6 months after the refractory judgement were 4.0 and 4.2 g/dl, for sorafenib group and 3.5 and 3.4 g/dl, for TACE group, respectively ($p = 0.0693$ and 0.0578 , respectively).

Conclusions: Sorafenib conversion might prolong TTDP and improve the serum albumin in TACE-refractory patients with intermediate-stage HCC.

*LBP-063***A case of resection for complete spontaneous regression of hepatocellular carcinoma**

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The patient was a 74-year-old man with a history of alcoholic hepatitis referred to our hospital for suspicion of hepatocellular carcinoma (HCC). On abdominal echo findings, low echoic mass was detected in the segment 8 of the liver in March 2015. The tumor had got bigger for 3 months on contrast-enhanced computed tomography (CT) image, the tumor maximal diameter was 36 mm in size and was well-enhanced in the arterial phase and washed out in the portal and delayed phase. The serum alpha fetoprotein level was increased in 30.8 ng/mL and the percentage of the L3 isoform was 25.5 %. The tumor was detected as isoform-low density lesion and had got smaller, the maximal diameter was 16 mm in size, and contrast enhancement of the tumor was disappeared on CT finding after 1 month. Spontaneous necrosis of the tumor was considered, but we could not exclude viable malignant cells in the tumor. Segmentectomy of S8 was

performed. In the resected specimen, the tumor had a thick fibrous capsule. Histopathological findings only showed granulation tissue and necrotic tissue, accompanied by bleeding and hemosiderosis. No viable tumor cells were observed. The serum alpha fetoprotein level returned to normal range 1 month after surgery. From the preoperative images, the decrease of the tumor marker, and the pathological findings, our case was diagnosed as complete spontaneous necrosis of hepatocellular carcinoma. Spontaneous complete necrosis of hepatocellular carcinoma without any pretreatment or angiography is extremely rare.

*LBP-064***Surgical outcome of hepatocellular carcinoma with intraatrial thrombus in Child B cirrhotic patient**

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The prognosis of the hepatocellular carcinoma with intraatrial tumour thrombus was very poor. Surgical resection had been demonstrated to improved overall survival. However, the extensive surgery has been limited only in the Child A liver cirrhotic patients. We presented a 39-year-old man who had HBV liver cirrhosis Child B and huge hepatocellular carcinomas over the left lobe of liver with middle and left hepatic vein tumour thrombus and extended into IVC and right atrium. We demonstrated extended left hepatectomy with intraatrial tumour removal under beating heart cardiopulmonary bypass without any post-operative complication. After the hepatic venous outflow obstruction had been removed, the hepatic function has been improved from Child-Pugh score 9–6 (Child A) and the patient was discharge on the 10th postoperative day. The IVC and atrial tumour extension can cause venous outflow obstruction of the liver which produce deterioration of the liver. Surgical resection can be improved hepatic function. However, the long term benefit remain uncertain.

*LBP-065***Elevated A20 gene expression in PBMCs is associated with acute-on-chronic hepatitis B liver failure**

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Aberrant immunity contributes to the pathogenesis of acute-on-chronic hepatitis B liver failure (ACHBLF), and A20 is a newly identified negative regulatory molecule of the immune response. However, no data have been reported for the role of A20 in ACHBLF. This study aimed to investigate A20 mRNA expression in ACHBLF and to determine the potential of A20 as a biomarker for the prognosis of ACHBLF. Quantitative real-time polymerase chain reaction (qPCR) was used to measure the mRNA expression of A20 in peripheral blood mononuclear cells (PBMCs) from 137 ACHBLF patients, 105 chronic hepatitis B (CHB) and 35 healthy controls (HCs). A secondary cohort with 37 ACHBLF patients was set up as

validation data set. The plasma levels of interleukin (IL)-1 β , IL-6 and IL-10 were determined using enzyme-linked immunosorbent assay (ELISA). Receiver-operating characteristic (ROC) curves were used to determine the predictive value of A20 for the prognosis of ACHBLF patients. A20 mRNA expression in ACHBLF was significantly higher compared with CHB and HCs. In ACHBLF patients, A20 mRNA was closely associated with total bilirubin, albumin, international normalized ratio, prothrombin time activity and model for end-stage liver disease. Furthermore, A20 mRNA was significantly correlated with IL-6 and IL-10. An optimal cut-off value of 12.32 for A20 mRNA had significant power in discriminating survival or death in ACHBLF patients. In conclusion, our results suggest that the up-regulation of the A20 gene might contribute to the severity of ACHBLF and A20 mRNA level might be a potential predictor for the prognosis of ACHBLF.

LBP-066

GRP78 is an antiviral factor and one of the antiviral targets against hepatitis A virus replication

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Background and aims: Hepatitis A virus (HAV) infection is one of the major causes of acute hepatitis in the world, and could occasionally lead to acute liver failure. So, it is important to unravel the further pathogenesis of hepatitis A. To investigate the association between HAV replication and endoplasmic reticulum (ER) stress marker glucose-regulated Protein 78 (GRP78/Bip) expression.

Materials and methods: (1) Huh7 was transfected with GRP78-siRNA (siRNA against GRP78) or control-siRNA using electroporation. (2) Huh7 was transfected with GRP78 CRISPR/Cas9 ko plasmid. After 48 h of transfection, monoclonal selection of cell was performed. Huh7-GRP78 knock-out (ko) were confirmed by Western blotting. (3) Cells were infected with HAV HA11-1299 genotype IIIA strain. After 96 h of infection, HAV RNA levels were measured by real-time PCR.

Results: (1) HAV RNA was upregulated in Huh7 cells transfected with GRP78-siRNA than that in Huh7 cells transfected with control-siRNA (1 vs. 8.7 ± 0.085). (2) HAV RNA was upregulated in Huh7 cells transfected with GRP78 CRISPR/Cas9 ko plasmid than that in control Huh7 cells (1 vs. 13.7 ± 0.13). (3) In Huh7 cells transfected with GRP78 CRISPR/Cas9 ko plasmid, ATF6 and XBP1 expression were also down-regulated.

Conclusion: HAV infection was enhanced by knock-down or knock-out of GRP78. GRP78, one of the ER stress-associated proteins, was revealed as an intracellular anti-HAV factor using 2 loss-of-function studies, which suggested a new defense against HAV in hepatocytes.

LBP-067

HAV IgG seroprevalence among HBV liver disease in Daegu city of South Korea

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Introduction: Coinfection of hepatitis A can aggravate liver damage in HBV liver disease patient. Vaccination against HAV was commenced in late 1990s. We need to know HAV IgG seroprevalence in HBV patient nowadays.

Method: We checked HAV IgG in 76 patients who have HBV from 2013 to 2015. Seroprevalence was analyzed retrospectively.

Result: Mean age was 40 years old. Men to women ratio is 55:45(42/34). Seropositivity of IgG is 65 % (50/76). (1) According to age, 10–19 years 12 % (1/8), 20–29 years 25 % (1/4), 30–39 years 33 % (6/18), 40–49 years 85 % (17/20), 50–59 years 93 % (13/14), 60–69 years 100 % (10/10), and 70–89 years 100 % (2/2). Seropositivity of IgG is increasing as age becomes higher ($p < 0.05$). (2) Seropositivity of patients below 40 years old is 26 % (8/30) and above 40 years old is 91 % (42/46). Seropositivity is high in patient above 40 years old ($p < 0.05$). (3) Seropositivity of patients in man is 60 % (25/42) and women is 62 % (20/32). Seropositivity between men and women is not different ($p > 0.05$).

Conclusion: Seropositivity of HAV IgG was not high in HBV young generation. We need to consider test and vaccination. HBV young generation can be prevented from coinfection of HAV.

LBP-068

Hepatitis E virus in patients with HIV infection

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Introduction: Hepatitis E virus (HEV) infection is an emerging infection in developed countries and is thought to be a porcine zoonosis [1]. We are study the incidence of chronic HEV coinfection in patients with HIV infection.

Materials and methods: A total of 246 patients with HIV infection and 94 control subjects were tested for HEV using an immunoassay for anti-HEV IgG and were tested for anti-HCV and HBsAg.

Results: The prevalence of HEV IgG seropositivity in the 246 HIV infection is shown that in the male group, 19.1 % (27/141) were positive as against 29.5 % (31/105) in the female group. The prevalence of HEV IgG seropositivity was significantly higher in women than in men ($P < 0.05$). In addition, subjects over 40 years of age had a higher prevalence of HEV IgG seropositivity than those aged >40 years (OR = 2.780, $P < 0.01$). There was no difference in anti-HEV IgG seroprevalence between the HIV-infected patients and controls.

Conclusions: Anti-HEV seroprevalence is similar in controls and patients with HIV infection. Risk factor analysis suggests that HEV is not transmitted sexually. No statistically significant association between HEV seropositivity and HBV and HCV infection was observed.

Reference: 1. Hoofnagle J.H., Nelson K.E., Purcell R. H. Hepatitis E. The New England Journal of Medicine. 2012;367(13):1237–1244.

LBP-069

Total anti-HBc/IgM Anti-HDV ratio helps to identify patients with severe HDV-induced liver disease

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Background/aims: The development of reliable quantitative assays for viral serum markers prompted a significant improvement in the management of HBV-carriers. We assessed the clinical significance of the combined quantitative evaluation of HBV/HDV viral markers in a cohort of patients with chronic Delta-hepatitis.

Methods: Single-time-point sera from 122 consecutive HBsAg, anti-HDV, Gen-1-HDV-RNA positive patients (71 males; mean age, 46.1 ± 12.7 years; 10 HBeAg positive), enrolled in two Hepatology Centres (Italy and Romania), were tested quantitatively for HBsAg (Architect-assay, Abbott, USA), total-anti-HBc (Wantai, China), HBV-DNA (COBAS-TaqMan, Roche, Germany), HDV-RNA (end-point-dilution in-house PCR) and Anti-HDV Antibodies (HDV IgM and HDVAb Assays, Dia.Pro srl, Italy).

Results: HBV markers levels correlated between them but not with HDV-markers: HBsAg (IU/mL) with HBV-DNA (IU/mL) [$\rho = 0.202/p = 0.033$] and Anti-HBc (IU/mL) [$\rho = -0.204/p = 0.025$]. Anti-HBc levels were significantly lower in cirrhotics ($p = 0.003$) whereas HBV-DNA and HBsAg did not correlate with any clinical, laboratory and pathologic feature. IgM-anti-HDV (AU/mL) correlated with total anti-HDV (terminal-dilutions) [$\rho = 0.622/p < 0.001$], AST [$\rho = 0.486/p < 0.001$], ALT [$\rho = 0.454/p < 0.001$], GGT [$\rho = 0.283/p = 0.002$], AFP [$\rho = 0.305/p = 0.003$], PLT [$\rho = -0.247/p = 0.007$], Fibroscan [$\rho = 0.271/p = 0.008$]. Since anti-HBc were significantly higher in chronic-hepatitis than cirrhosis (3754.5 ± 8839.5 vs 1207.9 ± 3009.8 IU/mL, $p = 0.003$) whereas for IgM-anti-HDV was the opposite (36.9 ± 29.3 vs 62.2 ± 55.6 AU/mL, $p = 0.043$), we computed their ratio as indicator of severity of chronic Delta-hepatitis and found that cirrhotics had significantly lower values ($p < 0.001$). Overall, anti-HBc/IgM-anti-HDV-ratio correlated significantly with disease severity [AST: $\rho = -0.364/p < 0.001$; ALT: $\rho = -0.269/p = 0.003$; AFP: $\rho = -0.243/p = 0.019$; Fibroscan: $\rho = -0.228/p = 0.026$]. The ROC-curve analysis (AUROC: 0.708, $p = 0.001$) identified the cut-off of 18 with 64.2 %-sensitivity and 70.4 %-specificity.

Conclusion: Our study identifies the quantitative total-anti-HBc/IgM-anti-HDV ratio as potential indicator of the severity of chronic Delta hepatitis and prompt prospective studies to test it in clinical practice.

LBP-070

Perihepatic nodes by point-of-care ultrasound in acute hepatitis and acute-on-chronic liver disease

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Aim: To study the manifestations of perihepatic lymph nodes during the episode of acute hepatitis flare by point-of-care ultrasonography.

Methods: One hundred and seventy-six patients with an episode of acute hepatitis flare were enrolled retrospectively. Diagnosis of etiology of the acute hepatitis flare was based on chart records and serological and virological assays. The patients were categorized into two groups (viral origin and non-viral origin) and further defined into ten subgroups according to the etiologies. An ultrasonography was performed within 2–72 h. The maximum size of each noticeable lymph node was measured. Correlation between clinical parameters and nodal manifestations was analyzed.

Results: Enlarged lymph nodes were noticeable in 110 (62.5 %) patients, mostly in acute on chronic hepatitis B (54.5 %). The viral group had a higher prevalence rate ($89/110 = 80.9$ %) and larger nodal size (median, 7 mm) than those of the non-viral group ($21/66 = 31.8$ %; median, 0 mm). Meanwhile, there were significant differences in the nodal size between acute and chronic viral groups, and between acute hepatitis A and non-hepatitis A viral groups. In logistical regression analysis, the nodal width still showed strong significance in multivariate analysis to stratify the two groups. The area under the curve of ROC was 0.805, with a sensitivity of 80.9 %, a specificity of 68.2 %, positive predictive value of 80.92 %, negative predictive value of 68.18 %, and an accuracy of 76.14 %.

Conclusion: Point-of-care ultrasonography to detect perihepatic nodal change is valuable for clarifying the etiologies in an episode of acute hepatitis flare.

Cut-Offs	AFP				PIVKA-II				AFP+PIVKA-II
	>10	>20	>100	>400	>40	>48	>70	>150	
HBV									>10; >70
Sensitivity	36.4	29.5	20.5	18.2	75.0	68.8	58.3	39.6	65.9
Specificity	97.1	100	100	100	76.5	85.3	94.1	94.1	91.2
DA	62.8	60.3	55.1	53.8	75.6	75.6	73.2	62.2	76.9
HCV									>100; >48
Sensitivity	69.6	51.3	23.5	11.3	76.2	71.4	60.3	49.2	74.8
Specificity	44.4	75.6	95.6	100	57.1	69.4	73.5	91.8	66.7
DA	62.5	58.1	43.8	36.3	70.9	70.9	64.0	61.1	72.5
Non-Viral									>100; >70
Sensitivity	36.7	24.1	17.7	10.1	84.5	81.0	73.8	57.1	77.2
Specificity	94.1	100	100	100	56.8	59.5	75.7	83.8	73.5
DA	54.0	46.9	42.5	37.2	76.0	74.4	74.4	65.3	76.1

*LBP-071***Changing clinical profile of liver enzyme abnormalities among HIV-infected persons****Ashok R. Mohite, Pravir A. Gambhire, Sunil Pawar, Samit S. Jain, Ravindra Surude, Qais Q. Contractor, Pravin M Rathi**

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Background: As the survival of HIV infected patients is increasing, liver disease is being recognized as a major issue. Data about the etiology of deranged liver function tests (LFTs) is mostly from western countries.**Aims and objectives:** To study the clinical spectrum and identify factors associated with abnormal LFTs in HIV infected patients.**Materials and methods:** This was a cross sectional, prospective study in a tertiary care hospital. Ninety-eight HIV infected patients with deranged LFTs were evaluated for possible causes and outcome. They were compared with an equal number of HIV positive and HIV negative patients with normal LFTs.**Results:** Cause of liver dysfunction was established in 86/98 HIV infected patients with deranged LFTs. Most common causes of deranged LFTs were drug induced (anti-tuberculosis and anti-retroviral therapy) 29 %, chronic hepatitis B 18 %, alcoholic liver disease 11 % and non-alcoholic steatohepatitis (NASH) 7 %. Portal biliopathy (4 %), granulomatous hepatitis (2 %) were seen in few patients. Raised serum triglyceride, serum cholesterol, low CD4 count and long duration of HIV were significant in the deranged LFT group. Eight patents died during the 6 month follow up. Mortality due to liver disease was high in HIV infected patients with increased serum bilirubin and white blood cell count and reduced hemoglobin and total serum protein.**Conclusion:** Spectrum of liver disease among HIV infected patients is changing. In the era of Highly Active Antiretroviral Therapy opportunistic infections are reducing and deranged LFTs are usually due to drug induced liver injury, alcohol, NASH and chronic hepatitisB.*LBP-072***Initial experience with a biodegradable stent on the management of a Benign bile duct stricture****Katrina Jewel S. Cham, Ermil R. Magsino, Arlinking O. Go, Evan G. Ong**

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Significance: Bile duct stricture is an uncommon but challenging clinical condition that requires a multidisciplinary approach. Benign biliary strictures constitute about 25 % of all biliary strictures. Surgical operations, such as cholecystectomy, are the most common causes. Over the last two decades, advances in ERCP and therapeutic accessories have made endotherapy a treatment option for even the most complicated biliary strictures. However, biliary dilation is extremely painful and repeated ERCP is expensive and associated with significant morbidity. Clinical Presentation and Management: A 58-year-old female developed post-surgical stenosis of the common hepatic duct (CHD) after a difficult laparoscopic cholecystectomy which required insertion of a left intrahepatic drain. Two months post-operatively, she developed jaundice, fever and abdominal pain. ERCP after removing the drain revealed a short stenosis at the junction of

CHD and proximal common bile duct requiring placement of a 7 French plastic stent. However, after 2 months there was recurrence of cholangitis. A 10-cm long 10-mm fully covered Ella DV Biodegradable Biliary Stent was deployed with a double pigtail stent within to prevent migration. A repeat ERCP after 2 months showed disappearance of the expandable stent and an intact double pigtail stent. The previously known stenosis appeared significantly wider on cholangiography. There was good egress of contrast and easy passage of balloon extractor through the affected segment. Recommendation: Biodegradable stents that disintegrate over a defined period of time addresses the limitations of permanency, repeated ERCP and long-term complications associated with both plastic and expandable metal stents.

*LBP-073***Wire-guide saving ERCP rendezvous technique****Katrina Jewel S. Cham, Lord Byron C. Corral**

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Background/aims: Rendezvous technique guided ERCP usually achieves an almost 100 % cannulation rate in patients with percutaneous biliary access and difficult ERCP. However, the guidewire inadvertently gets damaged by the retrieval snare during the procedure. In economically challenged countries, this would be considered quite expensive.**Methods:** Post-cholecystectomy patients with retained stones and indwelling T-tubes and referred for ERCP stone extraction were the subjects. For those with difficult or failed cannulation, the rendezvous technique was employed. Here we present four wire-guide saving modifications of the rendezvous technique. (1) Side by side cannulation: wire protrudes from papilla and this is used as a guide for cannulation using standard papillotome. (2) Reverse cannulation: Wire with or without a catheter exiting from the papilla cannulate a large bore catheter protruding from the duodenoscope. The wire is fed into the duodenoscope till it comes out of the working channel. (3) Kissing method: Catheter over wire protruding from papilla, wire withdrawn and catheter slowly pulled back while the papillotome tip follows it. (4) Guided needle knife papillotomy: guidewire-catheter assembly is inserted thru and positioned out side papilla and used as a chopping board for needle knife incision.**Results:** All four wire-guide-saving modifications of the rendezvous technique achieved successful cannulation.**Conclusions:** Modifications of the rendezvous technique can be done to spare the wire-guide.*LBP-074***Effects of UDCA on bile secretion in patients with cholestasis****Lei Li, Bing Li, Huiguo Ding**

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Objective: To evaluate the effects of UDCA on bile secretion in patients with Cholestasis.**Methods:** 48 patients with ENBD were divided to UDCA group (n = 36) and control group (n = 12). The patients of UDCA group, including common bile duct stone (n = 9), bile duct carcinoma (n = 7), PSC (n = 7), post operation of liver transplantation

(n = 13), take UDCA orally (250 mg tid) and the drainage of bile per day, serum level of TBIL, TBA, and gamma-GT, ALP at the first day before and 7th day after ENBD of two groups were recorded.

Results: Compared with control group, the drainage of bile per day of UDCA group was increased significantly at 3rd day (t = 2.461, p = 0.048), 4th day (t = 3.896, p = 0.021) and 5th day (t = 2.760, p = 0.034) after ENBD, especially in patients with common bile duct stone, bile duct carcinoma and post operation of liver transplantation; the serum level of and gamma-GT, ALP at the 7th day after ENBD was decreasing dramatically, especially in patients with common bile duct stone (t = 2.503, p = 0.038, t = 2.158, p = 0.045 respectively) and post operation of liver transplantation significantly (t = 2.805, p = 0.029, t = 2.461, p = 0.034 respectively).

Conclusion: UDCA increase the bile secretion in patients with different kinds of Cholestasis and improve the liver function.

LBP-075

Acute fatty liver of pregnancy mimicking acute cholecystitis

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Background: The acute fatty liver of pregnancy is a metabolic disorder seen in the 3rd trimester. This obstetric emergency can result in the death of the mother and the baby if not defined prior to the development of coagulopathy secondary to acute liver failure. We here in present a patient with acute fatty liver of pregnancy that was diagnosed prior to the development of coagulopathy.

Case report: A 32-year-old, 35-week pregnant women was admitted to the emergency department with nausea, vomiting and epigastric pain radiating to the right upper quadrant. On physical examination tenderness was present with palpation upon the right upper quadrant. Laboratory examination was as follows: AST243 U/L, ALT291 U/L, LDH892 U/L, direct bilirubin 2.9 mmol/L, total bilirubin 6.1 mmol/L, PT13 s; APTT34 s. Ultrasonography performed with the initial diagnosis of acute cholecystitis showed normal gall bladder. Heterogeneous-patchy distributed steatosis in the liver was noticed (Figures). The patient underwent an emergency cesarean section with the diagnosis of acute fatty liver of pregnancy. The high laboratory values declined slowly in the 3rd day postpartum and returned to normal by the 9th day postpartum.

Conclusion: Acute fatty liver of pregnancy can be fatal for the mother and infant if not detected before the development of coagulopathy. These patients present with findings similar to acute cholecystitis such as epigastric pain, nausea and vomiting. In these patients acute fatty liver of pregnancy should be especially kept in mind. Ultrasonography is helpful to rule out acute cholecystitis and to show the liver steatosis.

LBP-076

Novel sonar techniques for patients awaiting liver transplantation clinical and cost impacts

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Background and aim: Umbilical hernias and esophageal varices pose management dilemma in patients with cirrhosis, especially those awaiting liver transplantation. Complications of umbilical hernias in cirrhotic patients with Portal Hypertension include leakage, ulceration, rupture and incarceration thus leading to a higher mortality rate after surgical repair. Additionally patients need follow up for screening esophageal varices while on the waiting for liver transplantation, eventually is a burden on endoscopy units.

Patients and methods: The intra-abdominal portion of the esophagus of 650 patients who presented with manifestations of decompensated liver with MELD score less 14 were examined using standard two-dimensional ultrasound (US) to evaluate cost effectiveness, standard issues, and medical benefits using conventional US. Additionally 23 patients of those patients presented with Incarcerated Umbilical hernia.

Results: The overall effectiveness analysis of 650 patients yielded a 41 percentage cost standard benefit calculated to be 114,760 American dollars in a 6-month study. However the overall success to predict esophageal varices using 2D U/S was 95 % and to treat patients with incarcerated hernia was 73.9 %.

Conclusion: To our knowledge, this is the first ever report to describe cost effectiveness value of 2D U/S technique for Esophageal varices prediction and sonar guided manipulation for fixing incarcerated umbilical hernia. Thus, this described procedure can be either a safe alternative Screening Endoscopy/Interventional Surgery or at least as a bridge for later safe elective Endoscopy/Surgery in patients awaiting for liver transplantation.

LBP-077

The development of organ transplant program in Kazakhstan

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Background: As one of the dynamically developing countries in a Central Asian region, Kazakhstan established the Organ Transplant program with the Transplant Law and Regulations starting from 2009. **Aim:** To analyze available data after implementation of Transplant Program in Kazakhstan.

Results: Overall, more than 500 patients had undergone transplantations of kidneys, liver and heart for the last 3 years. Our national center became a pioneer in performing liver transplantation from a cadaveric donor since 2013, and first to perform pediatric transplantation. Starting from 2013 in collaboration with transplant centers from different countries living donor liver program was prioritized. Figure 1 illustrates the characteristics of organ transplants in Kazakhstan from 2003 to 2014. The most prevalent cause of ESLD among liver recipients were viral hepatitis B, D, C, and AIH, PBC. In a single center of Oncology and Transplant analysis the survival curve showed 1 death per group in cadaveric and living donor transplantation. Overall 12 transplants performed up to May 2015 (4—cadaveric, 8—living donors). The Kaplan–Meier curve is given in the Figure 2. One lethal case in LDLT was due to hepatic artery thrombosis (HAT) with

no available donor organ for re-transplantation. One recipient in DDLT died at early PO-period due to hemorrhage.

Conclusion: The development of Organ Transplant program in Kazakhstan is prioritized to decrease the mortality of patients with ESD on a waiting list. Also, a timely diagnosis of early PO- complications is necessary to prevent mortality in recipients from the single center analysis of our center.

LBP-078

Recurrent hepatitis C after liver transplantation is associated with post-transplant diabetes

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Background: The increased prevalence of post-transplant diabetes mellitus (PTDM) in hepatitis C patients is attributed to direct effects of HCV. However, the link between immunosuppression and recurrent hepatitis C for PTDM association is unclear. The objective of our study is to determine the incidence and risk factors of PTDM at NZLTU.

Methods: A retrospective study was performed on 134 HCV and 117 HBV (control) post transplanted patients at NZLTU between 1998 and 2013. Pre-transplant data included demographic, virologic data and features of metabolic syndrome. Post-transplant data included glucose levels at 3, 12 and 24 months, histological features, SVR, steroid usage and diabetes management.

Results: The prevalence of pre-transplant Diabetes Mellitus was similar in both groups. However, prevalence of post-transplant diabetes was significantly higher in HCV group (45 vs. 21 %; $p = 0.001$). 45(40 %) developed PTDM within 3 months and 50 % of them continued to have PTDM over the 24-month and current followup. Furthermore, prevalence of post-transplant hypertension and renal impairment was significantly higher in HCV group. Histological features, recurrent allograft hepatitis and SVR were not associated with PTDM in HCV group. The maintenance of immunosuppression was similar in diabetics and non-diabetics HCV Post-transplant patients. Overall, PTDM has no effect on survival outcome.

Conclusion: Transplantation for Hepatitis C is associated with PTDM at NZLTU, consistent with previous reports. The impact of DAA is yet to be seen on Insulin resistance.

LBP-079

Activation of YAP-1 by c-Myc is a therapeutic target in hepatocellular carcinoma under hypoxia

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Background and aims: The Hippo signaling pathway regulates organ size by controlling both cell proliferation and apoptosis via effectors such as yes-associated protein (YAP). Dysregulation of the Hippo pathway has been suggested as one of the therapeutic target in hepatocarcinogenesis. However, little is known about the role of YAP-1 in hepatocellular carcinoma (HCC), especially under hypoxic conditions. In this study, we investigated the role of YAP-1 in hepatocellular carcinoma (HCC) under hypoxic conditions.

Methods: The expression of YAP-1 was quantified using real-time PCR and immunoblotting. Human HCC cells (SH-03, SNU-475, SNU-761 and Huh-7) were grown under either normoxic or hypoxic conditions, either with YAP-1 siRNA or with control siRNA. MTS assay and invasion assays were performed to evaluate the role of YAP-1 in HCC. To investigate the signaling pathway responsible for the activation of YAP-1, immunoblotting was performed.

Results: The expression of YAP-1 was increased in HCC cells (SNU-761, SH-03, and Huh-7) under hypoxic conditions as compared to normoxic conditions. Suppression of YAP-1 using siRNA transfection resulted in significant decrease in tumor proliferation (SNU-761 and Huh-7) and increase in doxorubicin-induced tumor death, especially under hypoxic conditions. Oncogenic action of YAP-1 occurred via activation of the c-myc pathway, leading to up-regulation of unfolded protein response (UPR), including the 78-kDa glucose-regulated protein (GRP78/BiP) and activating transcription factor 6 (ATF-6), especially under hypoxic conditions.

Conclusions: These results indicate that activation of YAP-1 via c-myc pathway, which leads to activation of the UPR pathway, is a therapeutic target in HCC especially under hypoxic conditions.

LBP-080

Tacrolimus induced nephrotoxicity and pulmonarytoxicity in wistar rats

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Background and aims: Transplantation has evolved into an accepted treatment for end-stage organ failure. However it faces rejection because immune system recognizes the transplanted tissue as a foreign object. Immunosuppressant drugs are used to overcome this problem. Tacrolimus is an immunosuppressive drug which is utilized to minimize the risk of organ rejection. A research was designed to find out toxic effects of tacrolimus on lungs and kidneys.

Methods: Treated groups of wistar rats were established against control group. Each rat of experimental group was orally given the diluted solution of tacrolimus 3 mg/mL and dissected after 6, 12, 24 and 48 h respectively. Lungs and kidneys were excised, preserved and processed for hematoxylin and eosin and Prussian blue iron staining.

Results: Kidney tissues presented the signs of toxic effects on tissue architecture such as increased interstitial space, necrosis, glomerular shrinkage, increased Bowman's capsule space, acute tubular necrosis, dilated blood vessels and vacuoles formation in tubules. Lungs sections showed toxicity characterize by alveolar wall thickening, alveolar space collapsing, necrosis, interstitial round cell infiltrate and edema. Results of Prussian blue iron staining showed no iron deposition in kidney while in lungs sections iron accumulation was evident.

Conclusion: Taken together these observations we can conclude that immunosuppressive drugs may induce toxicity to certain extent with structural distortion of the tissue architecture.

LBP-081

Histopathological alterations in nervous, cardiac and pulmonary tissues induced by thioacetamide

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Background and aims: Thioacetamide (TAA) is a thionosulfur containing compound having wide industrial and laboratory applications, used as fungicide and to prevent orange decay. It is a necrogenic and carcinogenic agent used as model hepatotoxicant, due to toxic intermediate metabolites which can cause cellular injury. Present study involves the scrutinization of its effect on extrahepatic organs including brain, heart and lungs.

Methods: Two groups of wistar rats (*Rattus norvegicus*) weighting 175 ± 25 g were kept in fully aerated room and were supplied with normal rat chow and water ad libitum. A dose of 0.2 g/L of TAA in drinking water was given to the animals for 18 weeks, while control animals were given fresh water. Later on all animals were sacrificed and three extrahepatic organs viz brain, heart and lungs were collected and processed for hematoxylin and eosin staining for histological analysis.

Results: TAA causes mild necrosis, oedema, chromatolysis of nuclear material, gliosis and pyknosis in brain tissues, while cardiac tissues has signs of edema and hemorrhage. Lungs affected badly and show infiltration, necrosis, congested alveoli and edema.

Conclusion: TAA is not only a hepatotoxicant as it affects other tissues like brain, heart and lungs due to its carcinogenic and necrogenic potential.

LBP-082

Variations in red cell indices and protein profiling due to the exposure of metallic dust

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Background and aims: Industries of developing countries like Pakistan practice the use of protective gear hardly at low level. Wazirabad city of Pakistan is famous for its cutlery works. Multi metallic dust is generated in bulk in whetting units of cutlery industry where the stain less steel articles are grinded. Certain metals are carcinogenic and due to this reason the persons dealing with the grinding of cutlery items are at a risk of exposure remarkably. The purpose of this investigation was to reveal the effects of metal dust on certain hematological parameters and serum profiling of the workers of concerned industry.

Methods: Blood samples were drawn from healthy population of Lahore city (C), inhabitants of Wazirabad (W1 = no direct exposure) and the respective workers and categorized on the basis of exposure duration to metallic dust i.e. W-2, W-3 and W-4 on the basis of 1–13, 14–26 and 27–40 years of exposure to metallic dust respectively.

Results: Hematological analysis revealed statistically significant escalation in RBCs (p value = 0.0007), MCV (p value = 0.0027), HCT (p value = 0.0068) and RDW (p value = 0.0319) and a significant decrease of Platelet count (p value = 0.0004), in experimental groups as compared to control group. The serum profiling was done by SDS-PAGE of all the groups against protein marker of range 10–220 KDa has shown relative protein fractions from 73 to 287 KDa.

Conclusion: From the fluctuation in certain hematological parameters and increase in protein expression the presence of serious health hazards is concluded. Therefore, use of proper safety measures and education is strongly recommended.

LBP-083

In vitro hepatoprotective and anti-hepatitis B activities of *Cyperus rotundus* tuber fractions

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Liver diseases, the fifth most common cause of global death can be metabolic, toxin-induced or infective including hepatitis viruses. We investigated the in vitro hepatoprotective and anti-hepatitis B virus (HBV) therapeutic potential of *Cyperus rotundus* tuber organic (hexane, chloroform, ethyl acetate and n-butanol) and aqueous fractions. Of these, the n-butanol and aqueous fractions showed the most promising, dose-dependent hepatoprotection in DCFH-injured HepG2 cells at 48 h. DCFH-toxicated cells were recovered to about 88 and 96 %, upon treatment with n-butanol and aqueous fractions (200 mg/ml, each), respectively compared to DCFH-only treated cells. Further, *C. rotundus* fractions were tested for anti-HBV activities by measuring the expression levels of viral antigens (HBsAg and HBeAg) in the HepG2.2.15 culture supernatants. At 48 h post-treatment, the ethyl acetate, n-butanol and aqueous fractions showed dose-dependent inhibition wherein at a higher dose (100 mg/ml), HBsAg production was reduced to 60.27 %, 46.87 and 42.76 %, respectively. In a time-course study, HBsAg production was inhibited up to 50 and 40 % by ethyl acetate and n-butanol fractions (100 mg/ml, each), respectively on day 5. Three active fractions were further subjected to time-dependent inhibition of HBeAg expression, an indirect measure of HBV active DNA replication. At day 5 post-treatment, ethyl acetate and n-butanol fractions (100 mg/ml, each) downregulated HBV replication by 44.14 and 24.70 %, respectively. In conclusion, our results showed very promising hepatoprotective and anti-HBV potential of *C. rotundus* tubers fractions in vitro. Our data could therefore, provide the basis for the claimed traditional use of *C. rotundus* for jaundice and hepatitis.

LBP-084

Undiluted N-butyl-2-cyanoacrylate for obliteration of gastric varices: a single center experience

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Background: Approximately twenty percent of patients with portal hypertension eventually develop gastric varices (GVs). GV bleed less frequently than esophageal varices, but is usually more catastrophic. Treatment modalities differ depending on the type of GV and local expertise. N-butyl-2-cyanoacrylate (NBCA) has traditionally been used in combination with Lipiodol for obliterating gastric varices. However, prohibitive cost of the diluent Lipiodol prompted us to utilize undiluted NBCA for obliteration of gastric varices.

Methodology: All patients diagnosed to have gastric varices and underwent variceal obliteration using undiluted NBCA were included in the study. The sandwich method (saline-glue-saline) of injection using G 21 needle was employed in all cases. The patients' demographics, endoscopic findings, initial hemostasis, complications, rebleeding rates and bleeding related deaths were retrospectively reviewed.

Results: Initial hemostasis was achieved in 100 % of patients, while re-bleeding occurred in 20 % (2/10) of patients within 2 weeks.

Repeat glue injection were done in both cases without further bleed. There were no bleeding related deaths encountered. The only complication noted was minimal self-limited bleeding at the time of injection. The mean NBCA volume used was 1 ± 0.4 mL per patient session.

Summary: Use of undiluted *N*-butyl-2-cyanoacrylate is a safe, effective and economical alternative to the standard Glue-Lipiodol mixture in endoscopic obliteration of gastric varices.

LBP-085

Serum potassium concentration is associated with the initial effect of Tolvaptan to liver cirrhosis

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Background: Tolvaptan decreases body weight and improved intractable ascites and edema in patients with decompensated liver cirrhosis. The aim of this study was to clarify factors correlated to the initial effect of Tolvaptan.

Methods: There were 63 patients to whom Tolvaptan was administered in order to treat ascites or edema due to liver cirrhosis from November 2010 to July 2015. Among them, 37 patients achieved over 1 kg decrease of their body weight by 1 week from the start of Tolvaptan (group A), and the other 26 patients were assigned to group B. We compared characteristics between these two groups.

Results: Out of 63 patients, 34 patients (52 %) were male. Median age was 70 years. 33 patients (50.8 %) were HCV related cirrhosis. 37 patients (58.7 %) were Child Pugh grade C. 23 patients (36.5 %) had hepatocellular carcinoma. All patients had already received other diuretics. After the start of Tolvaptan, body weight decreased 2.1 kg on average by 1 week. In comparison with group A and B, serum potassium concentration was significantly lower in group A (3.4 and 4.2 mEq/L, $p = 0.005$). Multiple linear regression analysis revealed that lower serum sodium and higher serum creatinine had positive correlation with serum potassium ($R = 0.407$, $p = 0.005$).

Conclusion: Our study indicated that serum potassium may influence initial effect of Tolvaptan. Serum potassium is correlated with renal function, therefore we think that it is better to use Tolvaptan before renal function deteriorates by using large doses of other diuretics.

LBP-086

Hyponatremia is prognosis factor of refractory ascites

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Aim: To evaluate the prognosis factor in decompensated liver cirrhosis patients with or without further complications, such as hepatorenal syndrome and/or hepatocellular carcinoma.

Methods: Twenty-six patients (median age 67 years, males 20) with decompensated liver cirrhosis and refractory ascites were enrolled. All patients received diuretics (20–80 mg/day of furosemide and

50–100 mg/day of spironolactone). Furthermore we add Tolvaptan (7.5–15 mg/day for 7 days). The etiology of cirrhosis included hepatitis B (19 %), hepatitis C (46 %) and non B non C hepatitis (35 %). For analysis of prognosis, we perform multivariate analysis by cox proportional hazard model. Changes in the body weight were assessed. The serum sodium levels were also measured, and adverse events were recorded. A follow-up assessment was conducted 7 days after treatment with tolvaptan.

Results: Median survival time (MST) was 65 days. In multivariate analysis, prognostic factor was hyponatremia ($p = 0.037$, OR 0.278, 95 % CI 0.086–0.906). MST was 50 days in hyponatremia group ($n = 14$), 268 days in normal group ($n = 12$). The incidence of hyponatremia was 54 %. In patients with hyponatremia, the serum sodium levels increase after tolvaptan.

Conclusion: Hyponatremia is prognosis factor of refractory ascites in decompensated liver cirrhosis patients. Tolvaptan is a promising aquaretic for the treatment of hyponatremia in refractory ascites with decompensated liver cirrhosis patients.

LBP-087

Is spontaneous bacterial peritonitis still responding to third generation cephalosporins?

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Goals: The aim of this study was to assess the response of patients with SBP to third-generation cephalosporins in Nile delta.

Background: Current international guidelines recommend the use of a third-generation cephalosporin for empirical treatment of SBP. However, reports about antibiotic resistance for third-generation cephalosporins increased dramatically. Which raise the questions about these guidelines and if they are still valid.

Study: One hundred patients with liver cirrhosis, ascites and first episode of SBP signed informed consent to participate in this study. They were admitted to Department of Tropical Medicine and Infectious diseases, Tanta University Hospital for 1 week during the period from January to December 2014. The patients were randomly assigned to receive either cefotaxime 2 g every 12 h or ceftriaxone 2 g every 24 h. Their ascitic fluid samples were re-examined after 48 h and after 5 days to assess the response to third-generation cephalosporins.

Results: On assessment of the response to third-generation cephalosporins after 48 h, 65 % of patients had satisfactory response (decrease 25 % at least in PMNL count) while 35 % did not achieve a satisfactory response and were shifted to another antibiotic. After 5 days, only 46/65 of initial responders achieved complete response. Ceftriaxone had higher response rates than Cefotaxime. However, this difference was non-significant.

Conclusions: Based on our study, there is a dramatic decrease in the response rate of SBP to third-generation cephalosporins. This marked decrease in the response should be watched closely as it suggests re-considering of the current guidelines for empiric therapy in SBP.

LBP-088

Persistent spontaneous bacterial peritonitis in the patient with end-stage liver disease

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This case is a 37-year-old man, a chronic alcoholic, with chronic hepatitis B and cirrhosis of liver (Child-Pugh class C). He was admitted for the first episode of spontaneous bacterial peritonitis (SBP) and initially treated with intravenous cefotaxime. As ascitic fluid culture grew *Pseudomonas aeruginosa*, intravenous ceftazidime and amikacin were replaced according to the result of sensitivity test. After 1 week course of sensitive antibiotics, there were higher ascitic fluid polymorphonuclear leukocyte cell count and blood neutrophil leukocytosis. Follow-up analysis revealed higher ascitic lactate dehydrogenase (LDH) level than serum LDH, low ascitic glucose level and persistent growth of *Pseudomonas aeruginosa* in ascitic fluid without emerging resistant strain. Computerized tomogram of abdomen revealed portal vein thrombosis and lobulated ascites but no other evidence of intra-abdominal focus of infection. Then another 2 weeks course of intravenous meropenem and metronidazole, his clinical conditions and laboratory analysis were improved, hence he was discharged with oral ciprofloxacin. Literature were reviewed for discussion, persistent spontaneous bacterial peritonitis was rare complication in end-stage liver disease. Lower ascitic albumin level, higher SAAG, and higher MELD score at the time of diagnosis of SBP, were risk for PSBP. If symptoms, laboratory analysis, organism(s), or response are atypical, repeated paracentesis can be helpful in raising the suspicion of secondary peritonitis and prompting further evaluation and surgical intervention when appropriate. Prospective studies are needed to further explore the factors that predict treatment failure, and to investigate potential therapeutic measures for patients with persistent spontaneous bacterial peritonitis.

LBP-089

Clinical implications of muscle wasting in cirrhosis

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Malnutrition is a frequent complication in cirrhosis. Sarcopenia or muscle wasting is manifestation of malnutrition in cirrhotics. Sarcopenia results in cumulative declines across multiple physiologic systems characterized by impaired response to stressors, predisposition to poor outcomes and mortality. Numerous methods have been utilized to quantify body composition, however in patients with cirrhosis these methods were somehow limited by the presence of fluid retention and ascites. CT scan measurement of lumbar muscle is considered gold standard tool to quantify muscle mass and identification of sarcopenia. It could present an objective assessment of nutritional and metabolic condition of cirrhotic patients. In these retrospective study, we aimed to assess the prevalence of sarcopenia in cirrhotic patients and evaluate correlation between muscle wasting, liver impairment and mortality.

Figure 1 The endoscopic appearances of cirrhosis-associated polyps varied with size, shape, and location.

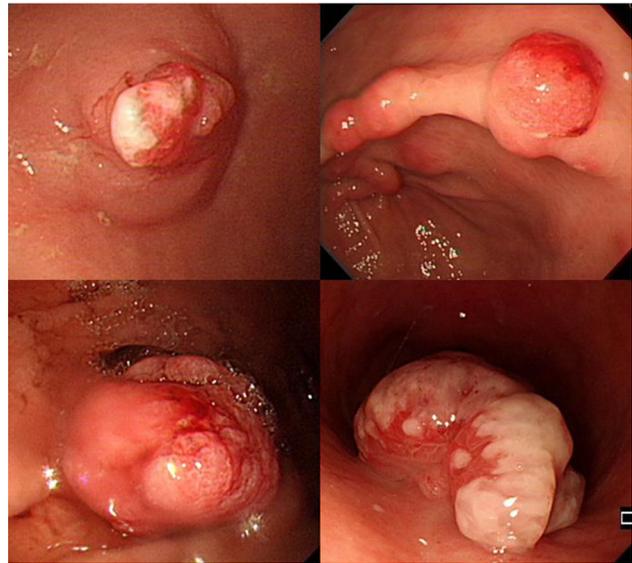


Table 1 The clinical characteristics of cirrhotic patients who received endoscopic polypectomy

	Bleeding	Non-bleeding	<i>p</i>
HBV	1(20%)	1(12.5%)	0.715
HCV	3(60%)	4(50%)	0.725
Child A	4(80%)	4(50%)	0.296
B	1(20%)	1(12.5%)	
C	0	3(37.5%)	
Alcoholism	1(20%)	2(25%)	0.835
EV	3(60%)	7(87.5%)	0.252
GV	1(20%)	1(12.5%)	0.715
Ascites	1(20%)	4(50%)	0.279
Platelet	128400±37447	114500±107054	0.788
PT prolong (sec)	1.16±1.56	1.68±2.11	0.649

LBP-090

Spontaneous splenic infarction in a cirrhotic patient

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Background: Spontaneous splenic infarction refers to a condition that oxygen supply of the spleen is interrupted due to occlusion of the splenic vascular or its branches, leading to parenchymal ischemia. The clinical spectrum ranges from asymptomatic disease, splenic abscess, to hemorrhagic shock. We presented one cirrhotic patients who diagnosed splenic infarction without usual etiologies. A 72-year-old female was previously diagnosed with cirrhosis and splenomegaly. She was admitted for fever and chills for 5 days. There were no abdominal pain, vomiting, anorexia. The abdominal echogram showed one 4.5-cm wedge-shape hypoechoic lesion in spleen.

The abdominal CT revealed one well-demarcated low-density area in spleen, favoring splenic infarction with consequent abscess formation. The patient didn't have evidence of hematological malignancy. The results of blood protein C, protein S, anti-nuclear antibody were normal. The EKG didn't display atrial fibrillation and echocardiography didn't demonstrated vegetations. After medical treatment, the size of abscess decreased and the patient was discharged smoothly.

Discussions: There are numerous etiologies of splenic infarction. The most common are hematological disorders, such as myeloproliferative disorders, leukemia, protein C or protein S deficiency, lupus anticoagulant, etc. Other malignancy and splenic infection (infectious mononucleosis, cytomegalovirus infection) may increase the tendency for clot formation. Some embolic disorders (endocarditis, atrial fibrillation) could form blood clots in circulation and cause splenic infarction. In our article, the patient didn't have evidences of above problems. She only complained fever and chills. We should keep in mind that splenic infarction is a relative rare disease but could happen in cirrhotic patients.

LBP-091

Gastric polypoid lesions in cirrhotic patients

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Background: Gastric hyperplastic polyps are inflammatory proliferations of the gastric foveolar cells. Many literatures suggested physicians to excise hyperplastic polyps larger than 1 cm. We found several unique characteristics about gastric polyps in patients with cirrhosis. The aim of this study is to elucidate the features and to analyze the clinical implications of gastric polyps in cirrhotic patients.

Methods: We retrospectively reviewed and analyzed 124 patients with gastric hyperplastic polyps confirmed by pathological examination at Mackay Memorial Hospital, Taitung Branch, from January 2010 to December 2014. We further discussed the prognosis of patients who had received endoscopic polypectomy.

Results: The endoscopic appearances of cirrhosis-associated gastric polyps varied, sessile or pedunculated, single or multiple, sized 0.5–3.0 cm (Figure 1). There were 13 cirrhotic and 42 non-cirrhotic patients who had received endoscopic polypectomy. Post-procedure bleeding rates were higher in cirrhotic patients than in non-cirrhotic patients (38.5 vs 11.9 %, $p = 0.030$). In patients with cirrhosis, the platelet counts were lower ($p = 0.000$) and the PT prolongation time were longer ($p = 0.015$) when comparison with non-cirrhotic patients. We further compare bleeding with non-bleeding groups. The results showed no significant differences between two groups regarding platelet count, PT prolongation time, HBV, HCV, Child Pugh score, ascites EV, GV (Table 1).

Conclusions: Cirrhosis-associated gastric polypoid lesions may have significant hazard of post-polypectomy bleeding. In this small size study, we didn't observe obvious factors to predict the risk of post-polypectomy bleeding in cirrhotic patients. The optimal management of these polyps requires established and may need further investigations.

LBP-092

Novel mutations in the CPT1A gene identified of carnitine palmitoyltransferase 1A deficiency

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Carnitine palmitoyltransferase 1A (CPT1A) is an enzyme functioning in mitochondrial fatty acid oxidation (FAO) of the liver. Patients with CPT1A deficiency have impaired mitochondrial FAO and display hypoketotic hypoglycemia and hepatic encephalopathy as typical manifestations. In this report, we present a case of CPT1A deficiency presenting jaundice as the first manifestation. A 1.9 years old boy showed jaundice and elevated levels of free and total carnitine were observed. From direct sequencing analysis of CPT1A, two novel mutations, c.1163 + 1G>A and c.1393G>A (p.Gly465Arg), were identified. At the age of 2.2 years, hypoglycemia, tachycardia, and altered mental status developed just after cranioplasty for craniosynostosis. High glucose infusion rate was required for recovery of his vital signs and mentality. Diet rich in high carbohydrate, low fat and inclusion of medium chain triglyceride oil resulted in improvement in cholestatic hepatitis and since then the boy has shown normal growth velocity and developmental milestones to date.

LBP-093

Serum M2BPGi and sodium level are associated with increased risk of mortality in patients with LC

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Background: Serum mac-2 binding protein glycosylation isomer (M2BPGi) has been recently revealed to be correlated with liver function reserve and with mortality in liver cirrhosis (LC). Hyponatremia is also reported as a predictor of death in decompensated LC. We evaluated the utility of M2BPGi and serum sodium level to predict all-cause mortality in patients with LC.

Methods: 166 consecutive patients with LC who visited our hospital during 2009–2010 were enrolled, after exclusion of patients with hepatocellular carcinoma (HCC) or with shorter than 1 month observation. We evaluated association between all-cause mortality and clinico-laboratory variables at entry.

Results: The study cohort consisted of 50.6 % male, with a median age of 62.3 years. 138 patients (83.1 %) were in Child-Pugh class A, HCV-Ab and HBsAg positive rate were 40.4 and 40.4 %, respectively. As 19 patients (11.4 %) died during the follow-up period (median 4.4 years), the cumulative survival rate was calculated as 89.5 % at 4-year. Multivariate analysis by Cox proportional hazards regression analysis identified M2BPGi > 4 [hazard ratio (HR) 12.90 (95 % confidence interval (CI) 2.87–58.04), $p = 0.001$], presence of hepatic encephalopathy [HR 5.48 (95 % CI 2.04–14.74), $p = 0.001$], and decrease in serum sodium concentration (per 1 mEq/mL) [HR 0.79 (95 % CI 0.65–0.96), $p = 0.016$] as independent risk factors for all-cause mortality.

Conclusions: Serum M2BPGi represents the noninvasive and objective method to assess the risk of clinical progression for patients with LC. Lower serum sodium level, despite being within normal ranges, may also influence the survival rate in patients with compensated LC, as well as to those with decompensated LC.

LBP-094

Complications profile of liver cirrhosis in Pakistan

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Complications of liver cirrhosis include hepatic encephalopathy, variceal bleeding, ascites and HCC. We report profile of these complications in our set-up.

LBP-095

Clinical characteristics of liver cirrhosis over 22 years in China

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The aim of this study was to describe the changes in cirrhosis etiology of liver cirrhosis. We conducted a medical record review of patients with liver cirrhosis from January 1993 to July 2014 in the Peking University First Hospital. Major clinical characteristics were abstracted and analyzed by cause of etiology, its accompanying disease, and its complication. There were 5067 patients with liver cirrhosis identified from the database with a mean age of 55.8 years (SD 13.4). The ratio of affected females to males was 1:2.7. The common causes of disease were: hepatitis B virus (HBV) or hepatitis C virus (HCV) 64.7 %, cryptogenic cause 13.9 %, alcohol 11.8 %, and autoimmune liver disease 10.9 %. The percentage of hepatitis B cirrhosis remarkably decreased from 69.8 % in 1993–98 to 46.4 % in 2009–2014 ($P < 0.001$), whereas the percentages of liver cirrhosis caused by alcohol and autoimmune increased substantially (from 3.0 to 15.3 %, $P < 0.001$; from 2.3 to 15.1 %, $P < 0.001$ respectively) over the same period. Ascites was found in approximately 50 % of patients with alcoholic cirrhosis, and kidney disease in 11.8 % of alcoholic cirrhosis patients.

Conclusions: Clinical features of liver cirrhosis in this population were changed dramatically over 22 years which were characterized by significantly decreased HBV-related cirrhosis while dramatically increased alcohol-related and autoimmune-related cirrhosis.

LBP-096

Rifaximin in the treatment of early hepatic encephalopathy

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Many patients with liver cirrhosis present with early hepatic encephalopathy, which includes impaired memory, mental confusion, disturbed sleep, disorientation, and agitation. In the absence of any obvious precipitating factors, like upper GI bleed, infection, drugs, and constipation, these patients are traditionally treated with lactulose to ensure effective bowel cleansing. We compared rifaximin and lactulose dual therapy with lactulose monotherapy, and found that dual therapy is better than monotherapy.

LBP-097

Severe vitamin D deficiency identifies a poor prognosis in patients with primary biliary cirrhosis

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Background: Increasing evidence suggests a close association between vitamin D and various chronic liver diseases. Our previous study showed that serum vitamin D levels of PBC patients are associated with disease severity and response to UDCA. We conducted this research to investigate the relationship of vitamin D level and long-term prognosis.

Methods: 98 consecutive PBC patients with frozen serum sample were reviewed. 25(OH)D levels were determined in serum samples collected before initiation of UDCA treatment. Long-term prognosis was defined as transplantation-free survival. Response to UDCA was evaluated by Paris-I criteria.

Results: The patients were followed up for a mean period of 65.2 months. Seven patients died due to liver related incidents or liver transplantation. The serum 25(OH)D levels in patients who suffered death or transplant were significantly lower compared to others (12.1 to 18.4 ng/mL, $p = 0.023$). The Kaplan–Meier curve showed a trend towards decreased transplantation-free survival among patients with vitamin D deficiency (less than 20 ng/ml), especially for those with serious deficiency (less than 10 ng/ml, $p = 0.016$). Besides, the patients who failed achieve complete response to UDCA had poorer survival than responders ($p = 0.031$). Furthermore, the patients who had low vitamin D level at baseline and failed to achieve complete response after 1 year of UDCA treatment got poorest transplantation-free survival ($p = 0.01$).

Conclusions: We conclude that vitamin deficiency is associated with advanced stages of PBC and it is a prognostic indicator for a poor long-term outcome.

LBP-098

Vitamin D supplementation therapy for primary biliary cirrhosis: a retrospective clinical study

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Background and aims: Previous studies suggest that vitamin D is associated with disease severity and response to UDCA in PBC. This research is designed to investigate whether vitamin D supplementation has an impact on response to UDCA treatment.

Methods: Consecutive PBC patients from Xijing Hospital between 2007 and 2013 were retrospectively reviewed. Basing on whether had received vitamin D supplementation during UDCA therapy, the

patients were divided into two groups. Response to UDCA was evaluated by Paris-I criteria. Logistic regressions were performed to identify the treatment response-associated parameters.

Results: Among 167 patients, 96 had a longtime vitamin D supplementation. There were no differences between two groups at baseline. After 1 year of UDCA therapy, the patients treated with extra vitamin D supplementation had a higher response rate than patients received UDCA monotherapy (68.8–52.1 %, $p = 0.029$). Risk factors for poor response to UDCA in univariate analyses include treatment without vitamin D, old age, late histological stage, and significant abnormal serum biochemical level. By multivariate analysis, treatment without vitamin D, stages III–IV and abnormal bilirubin value seems to be independently associated with incomplete response to UDCA.

Conclusions: Vitamin D supplementation may have a positive impact on responsiveness of UDCA in patients with PBC. Treatment without vitamin D is an independent factor associated with poor response to UDCA.

LBP-099

Experimental and clinical studies for development of anti-hepatofibrotic herbal drug, CGX

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Purpose: Liver fibrosis is the key pathological change which is arisen by most of chronic hepatic injuries. The progress of hepatic fibrosis determines the clinical outcome of patients, but no therapeutics for the disease exists yet. CGX is a modification of a traditional Korean herbal medicine, which has been used for patients suffering various liver diseases, including chronic viral hepatitis and alcoholic liver disorders.

Methods: The safety of CGX was evaluated in animal-based repeated toxicological studies using rats and beagle dogs. The pharmacological actions against hepatic fibrosis were evidenced in various chronic liver injury animal models using chemicals (CCl₄, DMN, or TAA), chronic alcohol consumption, choline-deficient (MCD) diet, and bile duct ligation (BDL) respectively.

Results: The main mechanisms of CGX related to anti-hepatofibrotic effects involve the inhibition of hepatic stellate cells producing extracellular matrix (ECM), down-regulation of pro-fibrogenic cytokines (TGF- β , PDGF, CTGF), and modulation of oxidative stressors and enhancements of antioxidant components. In addition, microarray experiment revealed the regulative action of CGX on VEGF gene expression. CGX is under clinical trial phase 3 for hepato-fibrosis therapeutic effect. The objective of the present study is to present the overall status for CGX development regarding its clinical backgrounds, pharmacological studies in animal models, and current process of randomized clinical trial.

Conclusion: It is now expected that multi-sites clinical trial evidences the fibro-therapeutic effects in patients with chronic viral or alcoholic liver diseases.

LBP-100

Transient elastography by fibroscan for the assessment of liver fibrosis: first study from Pakistan

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Background: FibroScan is the most popular non-invasive device used to assess hepatic fibrosis via transient elastography.

Objective: to analyze the hepatic fibrosis status by TE using FibroScan in patients with chronic liver disease according to underlying aetiology.

Materials and methods: This observational, descriptive and cross-sectional study was from September 2012 to August 2014. Patients with chronic liver disease, including CHB, CHC and NAFLD were included in the study to find out the degree of hepatic fibrosis.

Results: A total of 1122 patients with chronic liver disease were assessed. Overall, 76 % were males, mean age was 37 years. The most common aetiology was CHC followed by CHB. Mean liver stiffness measurement was 13.6 kPa.

Conclusion: CHC was associated with advanced fibrosis as compared to CHB and NAFLD.

LBP-101

Effect of ALT on transient elastography diagnosing liver fibrosis in chronic hepatitis B patients

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Background: Clinical practice indicated that elevated serum alanine aminotransferase (ALT) levels could result in unfaithful liver stiffness measurement (LSM) using transient elastography.

Aim: We carried out a multi-center study to validate the effect of ALT on transient elastography diagnosing liver fibrosis in chronic hepatitis B patients.

Methods: A total of 533 patients were enrolled. LS measurement and Liver biopsies were performed in all patients. Liver fibrosis stages were assessed according to Ishak criteria.

Results: Unqualified biopsy or unsuccessful LSM excluded 99 cases and 434 patients were analyzed. Multiple regression analysis indicated that LS value was independently associated with Ishak fibrosis stages ($P < 0.001$), ALT ($P < 0.001$), BMI ($P < 0.05$). For predicting fixed fibrosis stages, LS value was significantly increased with ALT levels ($P < 0.001$). As patients with rising ALT levels, performance of LSM for predicting fibrosis tended to decrease. In 111 patients with normal ALT, cut-offs of 9 and 6 Kpa recommended by ESAL guideline for with or without significant fibrosis could diagnosed 85 patients and discordant rate was 15.3 %; optimal cut-offs of 12.3 and 5.8 Kpa suggested in this study could diagnosed 65 patients and discordant rate was 4.6 %. In 271 patients with ALT 1–5 \times ULN, cut-offs of 12 and 6 Kpa recommended by ESAL for with or without significant fibrosis could avoid 139 biopsies and discordant rate was 15.1 %, this compared to 113 biopsies saved and 10.6 % discordant rate by optimal cut-offs of 15.5 and 6 Kpa.

Conclusion: Enhanced ALT levels could lead to overestimating LS values while predicting fixed fibrosis stages.

*LBP-102***Cause of cirrhosis in Mongolia evaluated by non-invasive methods including Fibroscan, FIB4 and APRI**

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Depending on the result of the Fibroscan, we made a cut-off point of 12 kPa to separate patients with F4 stage from F0, F1, F2, and F3 patients. To compare the result of the Fibroscan with other techniques we collected other laboratory results including AST level, ALT level, thrombocyte number, viral markers, and viral load. The above information was important for calculating FIB4 and APRI methods. Among 2758 people 57.7 % (1591) of people were HCV+, 35.7 % (984) of patients were HBV+, and 6.6 % (182) of patient were virus negative. Amongst 1590 patients who were HCV+, 62.4 % (992) of patients diagnosed with F4 stage of fibrosis by Fibroscan. On the other hand, 34.7 % (551) of patients with HBV+ has developed cirrhosis and 2.9 % (47) of patients had cirrhosis without any evidence of virus. In our further study, among 2.9 percentage patients with cirrhosis caused by non-viral etiology, 70 % were frequent alcohol consumers and only 15 % admitted that they were addicted to alcohol, and rest of the patients developed liver cirrhosis caused by other factors. This is the first time to determine exact causes of liver cirrhosis in Mongolia based on the large number of patients. We expected high efficiency of correlation between Fibroscan, FIB4, and APRI but the result was different than what we expected. In conclusion, the most common cause of liver cirrhosis is HCV, followed by HBV.

*LBP-103***ARFI is a useful tool in patients with chronic hepatitis B infection: a case report**

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A 60-year-old male patient with a previous diagnosis of hepatitis B virus (HBV) infection was seen at the outpatient ID clinic of our center. He was first diagnosed with HBV infection in 2008. Since then, the patient had been followed up with treatment for chronic hepatitis B (CHB) virus infection. In January 2012, the patient's laboratory study results were notable for HBVDNA level of 20,000 IU/ml, ALT:65 U/L, and trombocytes: 204,000/mm³. He underwent liver biopsy and findings were consistent with chronic hepatitis B [histological activity index (HAI) of 6/18, and grade 1/6 fibrosis]. He was followed up without antiviral therapy until February 2013, when an increase in his DNA levels (HBVDNA: 2,000,000 IU/ml) and a decrease in his trombocytes (140,000/mm³) was noted. Liver biopsy was performed to determine further treatment options. Biopsy findings showed grade 5/6 fibrosis and a HAI score of 9/18. Tenofovir at a dosage of 245 mg perorally daily was initiated. Since the rate of rapid progression of fibrosis levels in a 1-year period is usually low, the patient underwent acoustic radiation force impulse (ARFI) elastography examination for further evaluation. The median stiffness measurement was 1.1 m/s in concordance with fibrosis F1 in the region where the first biopsy was performed while the mean value

of the second measurement was 3.6 m/s in concordance with fibrosis F5 in the region where the second biopsy was done. This case deemed significant, as it showed that fibrosis was heterogeneously distributed throughout the liver in CHB patients and biopsy provided only a very small part of the whole liver. ARFI can serve as a guide for accurate collection of samples from identified regions of the liver.

*LBP-104***Utility of ARFI in the follow-up of a patient with chronic hepatitis B: a case report**

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A 27-year-old man visited ID outpatient clinic with complaints of weakness, fatigue and elevated liver enzymes. Poor oral intake was evident for 1 week. He was previously diagnosed with JRA at 2008. Until that time, he made regular visits to the outpatient physical medicine and rehabilitation department. He was first diagnosed with HBV infection in 2004. He was receiving corticosteroid and DMARD therapy. After 4 weeks of DMARD therapy his liver enzymes elevated 7 times to its upper limit. Liver biopsy was performed. In pathological examination; histology activity index (HAI) score was 7/18 and fibrosis score was found to be 3/6 according to ISHAC score. Entecavir 0.5 mg 1 × 1 therapy was initiated for the patient and follow-up visits at ID and physical medicine and rehabilitation department were scheduled. After 3 years initiating this therapy, his laboratory results were normal. Control liver biopsy was performed and histological improvement was observed (HAI:0/18, Fibrosis score: 0/6) in the pathological examination. Acoustic radiation force impulse (ARFI) elastography examination for further evaluation. In ARFI, The median stiffness measurement was 2.57 m/s in concordance with fibrosis F3–4 in the region where the first biopsy was performed while the mean value of the second measurement was 0.88 m/s in concordance with fibrosis F0–1 in the region where the second biopsy was done. ARFI would be a useful tool for both to initially assess patients and then as a monitoring tool to assess response to therapy. It may also serve as a useful guide before liver biopsy.

*LBP-105***Performance of acoustic radiation force impulse in patients with chronic hepatitis B**

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Aim: Hepatitis B virus infection is still one of the leading causes of cirrhosis and hepatocellular carcinoma. Liver biopsy is the gold standard method to assess the severity of liver fibrosis. However, there are several limitations of liver biopsy, including its invasive nature. Nowadays, noninvasive parameters have been utilized to

evaluate liver histology. There are only a few comparative study evaluating ARFI and liver fibrosis at the time of biopsy. The aim of this study was to investigate the relationship between ARFI technique and liver fibrosis in patients with chronic hepatitis B.

Materials and methods: 100 chronic hepatitis B patients and 30 healthy controls (130 individuals) were included in the study. ARFI measurement was performed all of these patients. Liver biopsy was done as a reference standard for 100 chronic hepatitis B patients. Liver biopsy samples were examined using the Ishak scoring system.

Results: Of the 130 cases, 107 (79.5 %) were male and 23 (21.5 %) were female. 30 of these cases were healthy controls and their fibrotic score is evaluated as F0. In segment 2, 3 and 8, there was a significant difference between ARFI measurements of the CHB patients according to different fibrosis groups ($p < 0.001$). Additionally there was a significant correlation between velocity measurements and fibrosis levels of the patients especially in segment 8.

Conclusion: ARFI is correlated with Ishak fibrosis score in CHB patients. ARFI would be a useful tool for both initially evaluating patients and then as a monitoring tool to assess response to therapy.

LBP-106

Early changes of liver stiffness in HCV patients undergoing treatment with DDAs: a multicenter study

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Introduction: The DDAs have been approved for advanced fibrosis and cirrhosis according to the results of transient elastography (TE) or liver biopsy. Aim of this study was to evaluate the early changes induced by DDAs on liver stiffness (LS). **Methods:** Four Italian centers enrolled 121 HCV patients (M 48.3 %, F 51.6 %; median age 65 years). All of them underwent TE at beginning and at the end of treatment: LS cut-off values for admission to treatment was >10 kpa.

Results: Patients were 26 and 74 % F3 and F4 respectively. The treatment was chosen according to the current guidelines. All patients cleared HCV-RNA by end of treatment. The average time between the end of therapy and TE was 79 ± 31 days. The mean LSM before and after treatment was respectively 23.9 ± 11 and 18.0 ± 10 Kpa ($p = 0.001$). Differences among LS values groups (10–17, 17.1–27.8, >27.8 kpa), evaluated by ANOVA, showed a statistically significant decline to 8.3 ± 8.0 kpa ($p = 0.039$), in LS >27.8 kpa group. Multivariate linear regressions analysis was utilized in order to evaluate the following variables associated to differences in LS: age, sex, BMI, baseline HCV-RNA load, antiviral therapy, bright liver, ALT, LS at baseline. The factors independently related to LSM decrease were: LS values at baseline ($p < 0.001$), HCV-RNA load ($p < 0.02$) and ALT ($p < 0.007$).

Conclusions: The early decline of LS at the end of treatment may be due to the rapid drop of necro-inflammatory activity. The evaluation on regression of fibrosis should require long term measurements of LS.

LBP-107

Egy-score can predict portal hypertension in chronic hepatitis C with good accuracy

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Background/aim: Egy-Score is a recently proposed non-invasive panel of biomarkers that predict stage of hepatic fibrosis and cirrhosis. We aimed to test the ability of Egy-Score as a predictor for the presence of portal hypertension and its complications in chronic hepatitis C (CHC) patients.

Methods: Treatment naive CHC ($n = 50$) patients we enrolled. They were classified into 2 groups (group A; with portal hypertension and group B; without portal hypertension) sub-classification of the two groups was done according to presence or absence of Bilharziasis. Patients were subjected to full clinical assessment, liver functions tests, HCV-Ab, HBsAg, ANA, Bilharzial agglutination titer, ultrasound abdomen and oesophago-gastro-duodensocopy. Egy-Score, AST-to-Platelet ration index (APRI) and FIB-4 were calculated according to their original publications.

Results: Our study included 50 patients (26 males) mean age was 55 years. Bilharziasis was detected in 13 patients (26 %), portal hypertension in 26 patients (52 %), esophageal varices in 23 patients (46 %) and ascites 19 in patients (38 %). Egy-Score was able to detect portal hypertension, esophageal varices and ascites with AUROCs of 0.929, 0.890 and 0.879 respectively. FIB-4 was able to detect portal hypertension, esophageal varices and ascites with AUROCs of 0.798, 0.750 and 0.752 respectively. APRI was able to detect portal hypertension, esophageal varices and ascites with AUROCs of 0.707, 0.657, and 0.708 respectively. Bilharziasis had no impact on the predictive ability of different scores.

Conclusion: Serum fibrosis biomarkers can predict presence of portal hypertension and its complications with good accuracy. Egy-Score was the best predictor among the studied biomarkers panels.

LBP-108

Utility of Sonazoid-enhanced ultrasonography for diagnosis of portal hypertensive gastropathy

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Objective: The aim was to elucidate the efficacy of contrast-enhanced ultrasound (CEUS) as a non-invasive diagnostic tool of portal hypertensive gastropathy (PHG) in patients with cirrhosis.

Materials and methods: This is a prospective study performed in 54 subjects, 40 cirrhosis patients (60.4 ± 10.5 years; male 29, female 11) who underwent the upper gastrointestinal endoscopy and measurement of hepatic venous pressure gradient (HVPG) and 14 controls (41.4 ± 13.1 years; male 10, female 4). Contrast enhancement at the upper stomach wall was assessed by the peak enhancement time (PT) and the intensity ratio between pre- and peak-enhancement (IR) using transabdominal CEUS with perflubutane microbubble agent.

Results: Fifteen cirrhosis patients had PHG (37.5 %), mild in 12 and severe in 3. The IR was significantly higher in patients with PHG

(1.67 ± 0.43) than in those without PHG (1.41 ± 0.24 , $p = 0.002$) and the controls (1.22 ± 0.17 , $p < 0.001$), although the PT did not differ significantly between them. There was no significant relationship between HVP and contrast-enhanced parameters (IR, PT) or the presence/absence of PHG. The area under the receiver operating characteristic curve of the IR was 0.8727 (95 % confidence interval 0.7673–0.9781) for the presence of PHG, with the best cut-off value of 1.7, sensitivity 64.3 %, specificity 88.5 %, positive predictive value 75 %, negative predictive value 82.1 %, and accuracy 80.1 %. Inter-operator variability for the intensity measurement was 6.3–11 %.

Conclusion: The CEUS may be promising as a non-invasive diagnostic tool for PHG in cirrhosis patients.

LBP-109

Non invasive diagnosis of esophageal varices: can it replace screening endoscopy?

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Goals: The aim of this study was to evaluate right lobe diameter of the liver/serum albumin ratio and other parameters as platelet count/spleen diameter, portal vein velocity and hepatic artery resistive index for accurate non-invasive prediction of oesophageal varices (OV).

Background: It is recommended that all cirrhotic patients should be screened for presence of OV. In developing countries, endoscopy screening of all cirrhotic patients is a large burden and compliance of the patient's decreases gradually.

Study: 100 cirrhotic patients were enrolled in the study. All patients were subjected to full history taking, thorough clinical examination, complete blood picture, complete liver functions, prothrombin activity, ultrasound on abdomen and pelvis, doppler US and upper endoscopy.

Results: Right lobe diameter of the liver/serum albumin could significantly predict OV with sensitivity 63.61 %, specificity 97.67 % PPV 97.3 %, NPV 66.7 % using a cutoff value 4.92. Platelet count/Bipolar spleen diameter could significantly predict OV with sensitivity 77.19 %, specificity 93.02 % PPV 93.6 %, NPV 75.5 % using a cutoff value 570. Portal vein velocity could significantly predict OV with sensitivity 85.96 %, specificity 86.05 % PPV 89.1 %, NPV 82.2 % using a cutoff value 12 cm/s. Hepatic artery resistive index could significantly predict OV with sensitivity 70.18 %, specificity 100 % PPV 100 %, NPV 71.7 % using a cutoff value 0.76.

Conclusions: Right liver lobe diameter/serum albumin, platelet count/spleen diameter and Doppler US parameters as portal vein velocity and hepatic artery resistive index should be performed in all cirrhotic patients as they are easy and can predict the presence of oesophageal varices.

LBP-110

CHI3L1 is a liver-enriched, noninvasive biomarker for staging and diagnosing hepatic fibrosis

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Liver fibrosis is a major disease that is primarily caused by hepatitis virus infections, toxins and alcohol abuse. Diagnosing and staging liver fibrosis are critical in guiding the treatment of chronic liver diseases, according to several international and Chinese guidelines. Liver biopsy is the gold standard for diagnosing and staging liver fibrosis, but it is invasive and suffers from several limitations. Consequently, much research has focused on the search for a noninvasive serum biomarker of fibrosis. In this study, we determined that Chitinase 3-like 1 (CHI3L1) is an abundantly expressed liver gene whose expression is highly enriched in the liver. We then compared serum levels of CHI3L1 among patients with different stages of liver fibrosis, as determined by liver biopsies, and found that the CHI3L1 levels were able to differentiate early stages of liver fibrosis (S0–S2) from late stages of liver fibrosis (S3–S4). We further showed that CHI3L1 is a good marker of substantial fibrosis, with areas under the ROC curves (AUCs) of 0.94 for substantial (S2–S4) fibrosis and 0.96 for advanced (S3, S4) fibrosis. Finally, we showed that CHI3L1 is superior to hyaluronic acid (HA), type III procollagen (PCIII), laminin (LN) and type IV collagen (CIV), which are also serum biomarkers of liver fibrosis, in identifying advanced liver fibrosis in patients with HBV-related liver fibrosis in China.

LBP-111

PDGF-BB as a biomarker for the assessment of liver fibrosis in patients with chronic hepatitis B

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Background: There were few studies investigating the circulating levels of Platelet-derived growth factor (PDGF), the most potent mitogen for hepatic stellate cells, in patients with chronic hepatitis B (CHB). The present study aimed to test whether PDGF-AA or-BB isoforms could serve as a potential biomarker for detection of liver fibrosis and thereby reduce the need for liver biopsy.

Methods: From October 2013 to August 2015, 465 patients with CHB were prospectively enrolled. All patients underwent liver biopsy and staging by Ishak system. Serum PDGF-AA and -BB were quantitatively measured by enzyme-linked immune sorbent assay.

Results: Serum PDGF-BB was negatively correlated with fibrosis stage ($P = 0.003$, Spearman $\rho = -0.16$) in all patients and has significantly difference between each fibrosis stage. PDGF-BB and PGT (combination of PDGF-BB and platelets) score were further developed to analyze the areas under the receiver operating characteristics curve (AUROC) of 0.667 and 0.829 for significant fibrosis in patients with normal ALT levels, comparable with AUROC of 0.823 and 0.821 for APRI and FIB-4 respectively. Importantly, using a cut-off of 0.45 with sensitivity of 0.67, specificity of 0.83, PPV of 0.60 and NPV of 0.84, 77.0 % of the cases with significant fibrosis in patients with normal ALT were correctly diagnosed by PGT score.

Conclusions: Serum PDGF-BB was remarkably decreased in fibrosis progression and could be used as a non-invasive biomarker for the assessment of fibrosis stage in patients with CHB. Furthermore, PGT score shown equal efficiency to other fibrosis scores in diagnosing significant fibrosis in patients with normal ALT.

LBP-112

Is diffusion MRI a useful tool for evaluating liver fibrosis?

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Aim: The aim of this study was to determine the apparent diffusion coefficient (ADC) values of liver fibrosis in diffusion MRI and investigate their role in detecting fibrosis.

Materials and methods: In the study a total of 43 patients (31 chronic hepatitis B, 7 non-alcoholic steatohepatitis patients, 5 patients were dismissed because of motion artefacts in images) were examined with 1.5 T, respiratory triggered DW-SS-EP MRI technique (b value of 1000 s/mm²). Patient's diagnosis were determined by means of histopathological evaluation. ADC values were measured in the lateral and medial segment of the left lobe, anterior and posterior segment of the right lobe. ISHAK score was used for pathologic grading of fibrosis.

Results: There was a significant difference between the ADC values of nonfibrotic and moderate-to-severe fibrotic liver of the patients. Also there was a significant difference between patients with mild fibrosis and patients with medium and advanced fibrosis. However, there were no significant differences between non fibrotic patients and patients with mild fibrosis. Also there were no statistically significant differences in terms of ADC values between patients with moderate and severe fibrosis.

Conclusion: The ADC values of moderate and severe fibrotic liver were found significantly lower compared to nonfibrotic and mild fibrotic liver of patients. Still, ADC values cannot take the place of percutaneous liver parenchymal biopsy.

LBP-113

Influence of soil heavy metals in fatty liver disease

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Aim: Although the association between ambient pollution with COPD and cardiovascular disease has been confirmed, the association between fatty liver disease and ambient pollution remains unknown. The study focuses on the novel risk factors of fatty liver disease.

Method: We correlated the severity of fatty liver disease with the level of soil heavy metals along with other blood biochemistry data, by using the residency provided in the records of the health evaluation center in Chang-Hua Christian hospital, and the data of soil metal concentrations from a nationwide survey conducted by Environmental Protection Administration of Taiwan.

Results: The prevalence of fatty liver disease diagnosed by *trans*-abdominal sonography in Taiwan is about 49 % according to our study population. Using univariate and multivariate analysis, we demonstrated that soil heavy metals is a significant risk factor of fatty liver disease in male, besides well-known risk factors like LDL cholesterol, BMI, or other metabolic risk factors. Among all the soil

heavy metals we have analysed, Chromium showed the strongest association to fatty liver disease.

Conclusion: The data indicated that there is some relationship between soil heavy metals and fatty liver disease. When we analyse by gender, the effect of soil heavy metals to fatty liver disease seems to be more obvious in male than in female.

LBP-114

Correlation between lipid and BMI with liver fibrosis in NAFLD patients

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Backgrounds: Non alcoholic fatty liver disease (NALFD) is one of common liver disease which the incidence was increasing in Asia Pacific including Indonesia last two decade. NALFD is one fatty liver disease that are not associated with alcohol consumption but related to high body mass index, dislipidemia and diabetes patients. NALFD will develop liver fibrosis and at the end will develop cirrhosis or hepatoma. Aim of study to know relationship between body mass index, lipid plasma with liver fibrosis by transient elastography (Fibroscan). Methods this was correlation study with cross sectional methods. We was collected NAFLD patients underwent liver transient elastography. Patients with chronic hepatitis B or C, alcoholic fatty liver and drug induced hepatitis were excluded from this study. Liver fibrosis were defined as 5.5 kPa or higher respectively using Fibroscan.

Results: Total of 19 male and 2 female were enrolled in this study. Mean age was 44.42 years, and mean body mass index was 27 kg/m². The highest level of liver transient elastography was 8.7 kPa and the lowest level was 3.9 kPa.

Conclusion: There are weak positive correlation between lipid plasma, and body mass index with liver fibrosis by liver transient elastography (Fibroscan).

LBP-115

The relationship between serum RPR and hepatic fibrosis in male nonalcoholic fatty liver patients

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Objective: To explore the relationship between serum RPR and hepatic fibrosis in male patients with nonalcoholic fatty liver disease.

Method: 120 male outpatients with nonalcoholic fatty liver disease and 20 healthy male control group were selected from the Affiliated Hospital of Yan'an University, the blood routine, alanine aminotransferase, aspartate aminotransferase and fibroscan value were tested, the liver fibrosis was also determined according to the BARD scores, then the results of testing and scoring were studied retrospectively.

Results: There was statistical significance of serum RPR index between the outpatients with nonalcoholic fatty liver disease and he

healthy control group; there were statistical significance of serum RPR index in the different BARD score groups; there were statistical significance of serum RPR index in the different hepatic fibrosis groups; the serum RPR index and fibroscan value were positively correlated in outpatients with nonalcoholic fatty liver disease.

Conclusion: It had correlation between the serum RPR index and hepatic fibrosis in outpatients with nonalcoholic fatty liver disease, the serum RPR index increased with the progress of liver fibrosis, monitoring the changes of RPR index is conducive to better monitor the progress of liver fibrosis, and to improve the accuracy of diagnosis of liver fibrosis. Monitoring the serum RPR index is helped for monitoring the progress of liver fibrosis, then improving the diagnostic accuracy of liver fibrosis.

LBP-116

Hepatic steatosis is associated with hepatocellular carcinoma in patients without viral hepatitis

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Introduction: Recent data suggest association between fatty liver and HCC. We postulate liver steatosis is a common association with HCC that develops in the absence of chronic viral hepatitis. We assessed the prevalence of steatosis in non-tumor liver in patients with non-viral HCC, and compared to that in patients with viral-related HCC.

Patients and methods: Surgical resection cases were identified from our pathology archives. Patients positive for virological and serological markers of HBV and HCV were excluded. Cohorts of age and gender matched HCC controls were also identified: (1) HBsAg negative and HBV PCR positive (n = 43). (2) HBsAg positive (n = 38). (3) HCV positive, but HBsAg and HBV PCR negative (n = 38). A hepatopathologist reviewed the slides to assess the grade of steatosis and the presence of steatohepatitis both in the tumor and in the background liver. Clinical data were also reviewed.

Results: Patients with non-viral HCC had a significant increased prevalence of steatosis and steatohepatitis in non-tumor liver, as well as steatosis and steatohepatic morphology within HCC. The grade of steatosis, both in the background liver and within HCC, was significantly increased (p < 0.001 and p = 0.04, respectively). There was no significant difference in the number of subjects with history or duration of excessive alcohol use (p = 0.163 and 0.795, respectively).

Conclusion: Hepatic steatosis is present almost three times the prevalence of hepatic steatosis in HCC associated with HBV, and close to twice that with HCV infection. This strong association suggests hepatic steatosis as a risk factor for non-viral related HCC.

LBP-117

Socioeconomic impact of ALCOHOL in patients with alcoholic liver disease (ALD) in Eastern India

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Background: There is no information on the social, economic, health and psychological impact of alcohol use in ALD patients and their families.

Aim and objective: To estimate the socioeconomic impact of alcohol use on patients with Alcoholic liver disease and their families in Odisha.

Methods: The demographic and socioeconomic data were collected from hospitalized ALD patients and Chronic liver disease patients of nonalcoholic etiology using dedicated questionnaire and analyzed.

Results: Study subjects included 400 consecutive CLD patients. 350 patients had CLD of alcohol etiology; 50 patients had CLD of non-alcoholic etiology (controls). In alcoholic group 80 % belonged to middle socioeconomic class; in control group, 82 % belonged to low socioeconomic group. Concomitant tobacco abuse was noted in 80 % in alcoholic group but 16 % in control groups. Average expenditure on alcohol was INR 2000/month. Average hospitalization for ALD related problems was 4.9 times/year with average expenditure of INR 30,000 per hospitalization compared to 4.6 times/year with average expenditure INR 45,000 per hospitalization in controls. For treatment expenses, 86 % (cases) and 94 % (controls) borrowed money from friends/relatives, 36 % (cases) and 18 % (controls) used saving deposits, 28 % (cases) and 22 % (controls) used state funds. Children were deprived of education in 43 % in alcoholic group compared to 14 % in control groups. 52 % (cases) and 08 % (controls) had disturbed social and family life. In alcoholic group 34 % abused family members, 20 % suffered accidents, 37 % indulged in physical violence, and 3 % attempted suicide.

Conclusions: Alcohol causes severe social disruption children are most to suffer Huge financial burden on both family and state.

LBP-118

Associations between vitamin D level and serum aminotransferase concentration in Korea

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Background: Recently, vitamin D has been recognized to have non-skeletal actions. The aims of this study was to investigate the association between vitamin D level and serum aminotransferase concentration in general Korean adults using the Korea National Health and Nutrition Examination Surveys.

Methods: Serum 25(OH)D levels were measured and then categorized into deficient (less than 20 ng/mL), insufficient (20–29 ng/mL) and sufficient (more than 30 ng/mL) groups. Elevated alanine aminotransferase (ALT) and aspartate aminotransferase (AST) concentration were defined as >30 IU/L for men and >19 IU/L for women. The association between vitamin D level and aminotransferase concentration were tested by Chi square tests and multiple logistic regression analyses.

Results: The prevalence of elevated ALT was 25.0, 24.7, and 22.6 % in deficient, insufficient and sufficient groups, respectively. The proportions of individuals with elevated AST were 30.0, 31.5, and 34.5 % in deficient, insufficient and sufficient groups, respectively. Compared to deficient group of vitamin D, subjects with higher serum vitamin D levels had significantly lower risks for elevated ALT concentration (insufficient group: adjusted odds ratio = 0.87, 95 % confidence interval 0.79–0.96, sufficient group: OR 0.76, 95 % CI 0.62–0.94) in the high-risk group for liver injury.

Conclusions: Higher vitamin D level was significantly associated with the lower risks of elevated ALT concentration in Korea.

*LBP-119***Clinical significance of focal hepatic solid lesions incidentally detected on ultrasonography****Nam Hee Kim¹, Yong Kyun Cho¹, Hong Joo Kim¹, Byung Ik Kim¹, Heon Ju Kwon²**¹Department of Internal Medicine, Kangbuk Samsung Hospital, Sungkyunkwan University School of Medicine, Seoul, Korea;²Department of Radiology, Kangbuk Samsung Hospital, Sungkyunkwan University School of Medicine, Seoul, Korea**Purpose:** The purpose of this study is to evaluate the clinical significance of focal hepatic solid lesions incidentally detected on initial ultrasonography in asymptomatic patients.**Methods:** From January 2009 to December 2009, 2670 initial ultrasonographies were performed in asymptomatic population. Of these 2670 initial examinations, 681 focal hepatic solid lesions in 542 patients were detected. Clinical information, ultrasonography features, and the outcome of these lesions were analyzed.**Results:** Six hundred and seventy-four lesions (99.0 %) in 539 patients (99.4 %) were benign, while seven lesions (1.0 %) in three patients (0.6 %) proved to be malignant. Risk factors significantly associated with malignant focal hepatic solid lesions were known history of malignancy, history of hepatitis, a positive result for the hepatitis B surface antigen, and abnormally elevated tumor markers. No malignancy was identified in patients without any one of these four risk factors. Ultrasonographic features of internal heterogeneous echotexture with peripheral hypoechoic rim showed significant associations with malignancy.**Conclusions:** Focal hepatic solid lesions incidentally detected on initial ultrasonography were rarely malignant, especially in patients without these risk factors. Therefore, the knowledge of these risk factors and US features is important in order to make a differential diagnosis between benign and malignant focal hepatic lesions.*LBP-120***Unbalanced distribution of medical resources for liver diseases in mainland of China****Hong Zhao, Jun Li, Yan Wang, Na Huo**

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To obtain the distribution of medical resources in diagnosis and management of liver disease in the mainland of China. Physicians who had been work for more than 1 year and take care of more than 10 patients with liver diseases weekly were eligible for the study. SAS software was used for the statistics analysis. Total 2803 eligible physicians finished questionnaire. Almost 67 % physicians worked in the Department of Infectious diseases in general hospital. In general hospital, there were 30.3 beds and 5.9 doctors per department. In specialized hospitals for the infectious diseases, 78 % of the physicians were in the department of liver diseases, the median number of beds and doctors were 43.3 and 5.3 per department. More than half of the physicians were female (54.41 %). Doctors' median age was 40 years. Nearly 60 % of physicians with senior professional title were male in the general hospital. Physicians believed that the first two obstacles in their career development were low income and non-harmonious doctor-patient relationship. Almost all the specialized hospitals could do HBV DNA level and serological test of HBV (94.3 and 98.3 %), compared with 86.65 and 81.6 % in general hospitals.

Much more specialized hospitals could do liver biopsy, 79.5 vs 52.9 %, $P = 0.00$. There were much more specialized hospitals had liver stiffness test (70.9 %). The allocation of medical resources in managing liver diseases in mainland China is not balanced. Much more HBV infection related tests and machines were in specialized hospitals and grade three of general hospitals.

*LBP-121***D.I.Y. dual purpose gastro-enteric tube (simultaneous gastric decompression and enteral feeding)****Ermil R. Magsino, Lord Byron C. Corral, Evan G. Ong**

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Enteral nutrition support through gastroenteric access plays a very significant part in the management of patients with compromised gastrointestinal tract. A 28-year-old female with history of intractable vomiting and abdominal pain came in for second opinion for gastric outlet obstruction. Index gastroscopy revealed a markedly edematous duodenum. There was diffuse thickening of the second portion of the duodenum on CT Scan. Histopathology revealed chronic duodenitis. Enteral feeding was contemplated but because of the unavailability of commercial and expensive dual-purpose and/or triple lumen feeding tubes, a self-fashioned gastro-enteric tube for simultaneous gastric decompression and enteral feeding was designed using readily available materials.

Methods: An 8 Fr feeding tube was tunneled thru a puncture along the side of an 18 Fr feeding tube until 30 cm of the smaller tube exits from the tip of the bigger tube. Multiple sideholes were also placed on the big tube proximal to the standard sideholes. Afterwards, the assembled feeding tube was inserted under endoscopic control with fluoroscopic guidance with the tips at the stomach (18 Fr NGT) and proximal jejunum (8 Fr NGT). Gastric decompression and jejunal tube feeding were concomitantly accomplished.

Results: The designed tube system satisfactorily achieved gastric decompression and drip jejunal feeding at the same time with marked clinical and nutritional improvement of the patient.

Conclusion: This self-fashioned nasogastro-enteric tube for simultaneous gastric decompression and enteral feeding can readily be used as an alternative to expensive and commercially unavailable triple lumen/dual-purpose feeding tubes.

*LBP-122***Hemochromatosis with a novel mutation of SLC40A1 gene in China: a case report****Jing Meng¹, Junqi Niu¹**¹The First Hospital of Jilin University, Changchun, Jilin, China; ²The First Hospital of Jilin University, Changchun, Jilin, China

Hereditary hemochromatosis predominantly affect Caucasians with a low incidence in Asians. Here we report the case of a 34-year-old Chinese male, who was admitted with hepatalgia, splenomegaly to our hospital. Hyperferritinemia of 12405 $\mu\text{g/L}$ with 50 % transferrin saturation of iron was noted in this patient. After excluding chronic hepatitis, autoimmune disorders, and alcohol or drug injury, genetic analyses of the patient and his mother revealed simultaneous manifestations of hereditary hemochromatosis, though his mother did not develop related symptoms. Liver biopsy showed his iron overload

was mainly in Kupffer cells of the liver. These subjects were heterozygous for c 485_487delTTG (Val162del), which was found as a novel mutation in the SLC40A1 gene in China. Now the patient is under treatment of bloodletting. These findings and the iron overload phenotype of the patient suggest that there are more patients need to be found.

LBP-123

Safety and efficacy of pyrazinamide in compensated cirrhosis: time to shun hepatotoxicity fear?

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Aims and objective: Patients with cirrhosis who acquire tuberculosis have a high hepatotoxicity risk during treatment with anti-tubercular drugs and generally have a poor prognosis. Pyrazinamide has traditionally been considered the most hepatotoxic anti-tubercular drug and not generally used in patients with cirrhosis. This study was performed to study the safety and efficacy of low dose pyrazinamide in patients with cirrhosis.

Methods: A randomized open labelled pilot study was performed in patient with compensated cirrhosis and tuberculosis. A total of 60 patients with compensated cirrhosis were enrolled from February 2012 to July 2014 and randomized to 2 treatment groups. Group A (n = 30) patients received isoniazid, rifampicin, levofloxacin and ethambutol for 2 months followed by isoniazid and rifampicin for 4 months, group B (n = 30) patients received low dose pyrazinamide (20 mg/kg) in place of levofloxacin. Safety and efficacy of both regimens was studied including hepatotoxicity development.

Results: The 6 months survival of patients was 98 %. The overall incidence of hepatotoxicity was 11.6 %. The incidence of hepatotoxicity was similar in both groups (p = 0.874). There was no difference between the group containing pyrazinamide or levofloxacin in terms of efficacy or survival.

Conclusion: The results of this study suggests that low dose pyrazinamide in compensated cirrhosis patients doesn't lead to additional hepatotoxicity and should be considered as a therapeutic option.

LBP-124

The correlation between HBV cccDNA and clinical features in children with CHB

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Background: To explore the relationships of intrahepatic cccDNA with clinical features of different phase in children with chronic hepatitis B (CHB).

Methods: A total of 60 children with CHB who had a liver biopsy were enrolled, including 20 in immune tolerate (IT) phase, 30 in immune activities (IA) phase, 10 in low-replicative (LR) phase. HBV cccDNA, serum HBs antigen and serum HBV DNA levels were detected.

Results: The median intrahepatic cccDNA levels were 6.73, 4.32 and 0.71 copies/cell for the patients with CHB IT, IA and LR phase,

respectively. HBV cccDNA was positively correlated with HBV surface antigen and HBV DNA level in the IA phase. HBV ccc DNA level was lower in CHB-LR than in the CHB IT and CHB IA. HBV cccDNA was positively correlated with histological activity index of liver inflammation in children with CHB IA phase.

Conclusions: Serum HBV surface antigen level may reflect the amount of intrahepatic cccDNA level in the IA phase of children with CHB.

LBP-125

Baseline factors predicting antiviral efficacy in children with HBeAg positive chronic hepatitis B

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Background/aim: To explore the predictive factors before IFN therapy on the virological response (VR) and serological response in children with HBeAg positive CHB.

Methods: 200 HBeAg positive children with CHB treated with IFN alone or in combination with lamivudine for 48 weeks were enrolled, serum levels of HBV DNA, HBeAg, ALT and HBsAg were determined at baseline and weeks 12, 24, 36 and 48. These patients were divided into two groups according to the levels of HBsAg, ALT and HBV DNA, respectively.

Results: Eighty percent patients achieved VR, comparison between the group with levels of HBsAg ≤ 1500 and ≥ 1500 IU/ml, the former showed that the loss rate of HBeAg/HBsAg were 64.3 vs 37.2 %, p = 0.007/50.0 vs 24.4 %, p = 0.005; the HBsAg seroconversion rate (35.7 vs 17.4 %, p = 0.025), but there was no difference in HBeAg seroconversion rate (39.3 vs 32.6 %, p = 0.484). Comparison between the group with levels of ALT $>ULN$ and $<ULN$, the loss rate of HBeAg/HBsAg (43.0 vs 23.8 %, p = 0.090/28.5 vs 23.8 %, p = 0.651); the HBeAg/HBsAg seroconversion rate (35.2 vs 19.0 %, p = 0.138/20.1 vs 19.0 %, p = 1.000). Comparison between the group with baseline levels of HBV DNA ≤ 108 and ≥ 108 IU/ml, there was significant difference in the loss rate of HBeAg (50.4 vs 28.7 %, p = 0.002) and undetectable rate of HBV DNA (82.9 vs 72.4 %, p = 0.019), but there was no difference in the seroconversion rate of HBeAg/HBsAg (38.9 vs 26.4 %, p = 0.063/24.8 vs 13.8 %, p = 0.054) and the loss rate of HBsAg (31.9 vs 23.0 %, p = 0.166).

Conclusions: Low baseline HBsAg level (≤ 1500 IU/ml) has high predictive value of VR and loss rates of HBeAg and HBsAg in the HBeAg positive children patients.

LBP-126

The effect of Myeloid derived suppressor cells in children with chronic hepatitis B infection

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Background/aim: To explore the expression level of Myeloid derived suppressor cells (MDSCs) in pediatric patients with chronic hepatitis B infection and the relationship with disease progress.

Methods: 30 pediatric patients with chronic hepatitis B (CHB) and the healthy children were enrolled. The peripheral blood specimens were collected and MDSC with CD33+CD11b+HLA-DR- were detected by flow cytometry. The difference of MDSC expression between the two groups were analyzed.

Results: The expression level of MDSC with CD33+CD11b+HLA-DR- in pediatric patients with CHB was significantly higher (1.61 ± 0.31) than in the normal control group (0.60 ± 0.27 , $p < 0.01$). During the antiviral therapy with interferon, the expression level of MDSCs with CD33+CD11b+HLA-DR- in the peripheral blood was significantly positive correlated with the decrease of HBV viral load and the aminotransferase in pediatric patients with CHB.

Conclusions: The proportion of MDSC in peripheral blood of pediatric patients with CHB is closely related to the occurrence and progression of disease, and may become a good clinical indicator for pediatric patients with CHB.

LBP-127

SCALFI ± Terlipressin mobilizes refractory ascites safely in decompensated liver cirrhosis

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Background: Refractory ascites mobilization is challenging.

Aim: Analyse efficacy and safety of low-dose, continuous, infusion of furosemide with albumin±Terlipressin in a response-guided protocol in refractory ascites to achieve complete mobilisation and $\text{UNa} > 80$ mmol/L.

Methods: Cirrhotic patients with refractory ascites were enrolled. Baseline blood, renal, ascitic fluid, examination including timed 24 h urine collection for urinary sodium (UNa), potassium (UK) were done. Furosemide infusion at 2 mg/h and albumin (20g/day) was started. Blood and urine (electrolytes) samples were collected 12 h and graded increase of furosemide was done by 1 mg (max 5 mg/h) and Terlipressin infusion @4 mg/12 h was added after 48 h with response guided increase (1 mg/12th h) if $\text{UNa} < 80$ mmol/L.

Results: 45 patients divided in 2 groups. Group1 had 25 (18 males) patients (Albumin-furosemide) and group2 (Albumin-furosemide-terlipressin) had 20 (19 males). Ascites responded to the treatment regimen in all patients over a median period of 7.4 ± 2.5 days. Group1: mean values of the patient were: age 47.2 ± 13.4 , CTP 11.48 ± 1.36 , MELD 24.2 ± 6.7 , creatinine at baseline (bl) 1.42 ± 1.35 , creatinine at end of treatment (EOT) 0.94 ± 0.43 , serum sodium (SNa) (bl) 129.28 ± 7.5 , SNa (EOT) 133.96 ± 5.45 , weight loss 9.44 kgs, UNa (bl) 17.2 ± 5.86 , maximum (max) UNa 171.24 ± 61.35 , UNa at discharge 86.4 ± 19.56 , serum albumin (bl) 2.5 ± 0.5 , urine output(UO) (bl) 642.7 ± 185.3 , UO (max) 2972.08 ± 803.6 . The mean values in group2 were: age 47.95 ± 9.35 , CTP 11.95 ± 1.27 , MELD 23.7 ± 6.8 , creatinine at baseline (bl) 1.89 ± 1.23 , creatinine (EOT) 1.47 ± 0.83 , SNa (bl) 132 ± 6.9 , SNa (EOT) 134.2 ± 7.5 , mean weight loss 11.55 kgs, UNa (bl) 16.8 ± 7.2 , max UNa 224.65 ± 169.45 , UNa at discharge 125.05 ± 57.03 , serum albumin (bl) 2.25 ± 0.5 , urine output (bl) 600 ± 257.3 , urine output (max) 4270 ± 2109.7 . In 2nd group 3 patients had severe abdominal pain, 4 had loose stools and 1 had t-wave inversion.

Sepsis and/or ACLF

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Bacterial infections are more frequent in patients with cirrhosis than in the general population. Cirrhosis-associated immune deficiency syndrome, an emerging concept, relates to a relative inefficiency of the innate and adaptive immune system in patients with cirrhosis to prevent or clear infectious agents in comparison with those without cirrhosis. Genetic predisposition for increased risk of infections in cirrhosis has been demonstrated with the *TLR2* GT microsatellite polymorphism and *NOD2* variants. A severely deregulated immune system that exists in patients with ACLF; from antigen processing to effector cell functions and cytokine release. The activated immune cells appear to be dysfunctional, paralyzed and energy-depleted. This situation results in an exaggerated SIRS, defective CARS and ultimately to an increased susceptibility to infections. Moreover, even in the absence of overt infection, patients with ACLF display sepsis-like characteristics such as reduced ex vivo TNF secretion, monocyte HLA-DR expression and increased IL-6 production. Any acute insult and ongoing hepatocellular injury, as seen in ACLF, would lead to an aberrant host inflammatory response, SIRS and infection. Occurrence and persistence of SIRS is associated with a hyperdynamic circulation, low mean arterial pressure, tissue hypoperfusion and leads to organ dysfunction in up to 40 % of patients with ACLF even in the absence of any overt infection. SIRS is associated with more severe encephalopathy, an increased incidence of bacterial infections, renal failure and poor survival than those without it. In a large study, the persistence of SIRS for up to 7 days or new-onset SIRS within the first week of hospitalization, correlated with progressive liver failure and high mortality (82 % with SIRS versus 48.7 % without SIRS; $P < 0.05$). Whether sepsis is a consequence or a cause of liver failure is not clear from the current data on ACLF. One viewpoint considers sepsis as a common extrahepatic precipitant of ACLF. However, liver failure is a late event in this group of patients with cirrhosis often concomitant with other organ failures and has a different clinical course. On the other side the concept that sepsis is a result of liver failure. Here it follows SIRS to sepsis in a time interval called window period. It proposes that prevention of SIRS or the development from SIRS to sepsis by immune modulation in this 'golden window' period could decrease the incidence of organ failure and improve survival. However large cohort studies including ACLF patients with infection at baseline and comparing them with those who develop infection subsequently could address this issue.

Non-invasive tests for liver fibrosis: EASL Clinical Practice Guidelines and future directions

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Over the past decade, the development of non-invasive methods to assess liver fibrosis has advanced the practice of Hepatology. The prognosis and management of chronic liver diseases greatly depend on the amount and progression of liver fibrosis with the risk of developing cirrhosis and its complications, including portal hypertension and hepatocellular carcinoma. Limitations of liver biopsy as well as availability of powerful viral tools and new antiviral drugs

have rapidly decreased the use of liver biopsy in viral hepatitis and led to the development of non invasive methodologies for the assessment of fibrosis. These methods rely on two different but complementary approaches: (1) a “biological” approach based on the dosage serum biomarkers; (2) a “physical” approach based on the measurement of liver stiffness using ultrasound-based elastography. The practical advantages of analyzing serum biomarkers include their high applicability (>95 %) and their potential widespread availability (non-patented). However, none are liver specific. Advantages of transient elastography (TE) include a short procedure time (<5 min), short learning curve, immediate results, and the ability to perform the test at the bedside or in an outpatient clinic. Although TE analysis has excellent inter- and intra-observer agreement, its applicability (80 %) is not as good as that of serum biomarkers, particularly in case of obesity or limited operator experience. Overestimation of liver stiffness values has been reported with several confounding factors including transaminases flares, extrahepatic cholestasis, congestive heart failure and food intake. Both methods perform better for cirrhosis than significant fibrosis. However, TE appears to be more accurate for detection of cirrhosis. Among novel techniques challenging TE, Acoustic Radiation Force Impulse imaging (ARFI) is the most validated, with accuracy similar to that of TE but better applicability. European Association for the Study of the Liver (EASL) Clinical Practice Guidelines on the use of non-invasive tests were released in 2015. The lecture of this session aims to review key recommendations from this publication with an emphasis on management of patients with viral hepatitis in whom these methods have been mostly validated.

Autoimmune liver disease: EASL clinical practice guidelines and future directions

Primary biliary cirrhosis (PBC), primary sclerosing cholangitis (PSC) and autoimmune hepatitis (AIH) have collectively been recognized as autoimmune liver diseases. PBC involves autoimmune damage to the small interlobular bile ducts causing non-suppurative destructive cholangitis. In PSC, autoimmune injury occurs at the medium to large bile ducts resulting in concentric fibrosis and biliary strictures. In AIH, self-reactive T and B cells target the hepatocytes, classically leading to a histological picture of interface hepatitis. The precise etiology and pathogenesis of PBC, PSC and AIH are poorly understood and available therapeutic strategies are only partially effective. Altogether autoimmune liver diseases comprise approximately 10 % of the overall European liver transplant program. The fraction of patients who develop progressive liver fibrosis under current treatment regimens varies, but lies within the range of 10–20 % for AIH, from 25 to 50 % in PBC and up to 100 % in PSC. Due to the limited level of evidence for any recommendation, establishing clinical practice guidelines (CPGs) in autoimmune liver diseases is challenging. Collectively, less than 75 randomized controlled trials have been performed in PBC, PSC and AIH (compared with e.g. 1000 in inflammatory bowel disease). European Association for the Study of the Liver (EASL) CPGs in PBC and PSC were released in 2009, whereas the EASL CPG for AIH was published toward the end of 2015. The lecture of this session aims to review key recommendations from these publications with an emphasis on difficult-to-treat patient categories. Moreover, key barriers moving toward therapies that are more effective will be elaborated. Although there is renewed optimism in the field following the initiation of a number of RCTs for novel drugs in PBC and PSC, study design in these trials poses considerable challenges and there is so far no robust consensus on what are the appropriate end-points. The principle approaches taken cover immune targets, novel bile acid derivatives, lymphocyte trafficking and anti-fibrotics. In preparing the audience for critical assessment of this

coming wave of trial results, the mechanistic basis for each of these approaches will be outlined along with main ongoing efforts to decipher the pathogenesis of these difficult-to-study liver diseases.

Treatment of hepatitis E

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No treatment for acute hepatitis E is required in the majority of cases as the infection clears uneventfully with the supportive care. However, hepatitis E virus (HEV) is an important cause of acute-on-chronic liver failure in the endemic areas and fulminant hepatic failure may occur during pregnancy. Chronic HEV genotype 3 infection and progressive disease has been reported in the recipients of solid organ transplants, hematological malignancies, HIV patients and those on hemodialysis. Clearance of HEV may occur after reducing immunosuppressive therapy in about one-third of the cases. Antiviral therapy should be considered for patients for whom immunosuppressive therapy cannot be reduced and for those who do not achieve viral clearance after reducing immunosuppression. The patients with severe infection, fulminant hepatic failure and acute-on-chronic infection should also be considered for ribavirin monotherapy to expedite the viral clearance and recovery. A 12 week course of pegylated interferon, ribavirin, or a combination of both, may clear virus in about two-third of patients with chronic hepatitis E. Three to twelve months treatment with pegylated interferon has been reported for the liver transplant recipients and patients on hemodialysis. In kidney and heart transplant patients where interferon may lead to organ rejection, ribavirin may be given. Sofosbuvir may be considered as an add-on therapy to ribavirin for the treatment of chronic HEV infection. There is no acceptable antiviral therapy for pregnant women.

IL28B and CHC

Jacob George

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Perhaps one of the landmark discoveries in liver disease and for hepatitis C in particular, as well as for personalized medicine in general, has been the discovery of polymorphisms in the interferon lambda (IFNL) region that regulates hepatic responses to HCV infection. This discovery using genome-wide association studies to examine the response to interferon-based HCV therapies has led to quantum improvements in our understanding of both the genetic basis and the underlying pathogenesis of HCV infection. In this context, the discovery of IFNL polymorphisms is unique with far reaching implications that extend well beyond HCV to various other liver and extrahepatic diseases. Three landmark GWASs on HCV were published almost simultaneously in the second half of 2009. The reports from the United States, Japan, and by our Australia-based collaborative group, demonstrated unequivocally that SNPs (rs12979860, rs8099917, rs12980275) in the IFNL region (formerly known as IL28B) were the strongest host factor associated with response to PegIFN/RBV therapy for chronic HCV genotype 1 infection. This finding has been replicated in other hepatitis C disease phenotypes, including infection with other genotypes and spontaneous clearance. More recently, it has been discovered that the original rs12979860 polymorphism is located in the first intron of IFNL4, and that several functional variants in the IFNL region exist. Porkunina-Olsson et al.

performed RNA sequencing of primary human hepatocytes activated with synthetic double-stranded RNA to mimic HCV infection and discovered a novel IFNL4. Production of the IFNL4 protein is dependent on the $\Delta G/TT$ (rs368234815) frameshift polymorphism and predicts HCV clearance better than the rs12979860 SNP, especially in African Americans. A second functional polymorphism in the 3' UTR region of IFNL3 (rs4803217) affects the mRNA stability of IFNL3. While the IFNL polymorphisms are less relevant in the era of direct acting, highly effective antiviral treatments for hepatitis C as a treatment response prediction tool, they have found new and exciting relevance in several other phenotypes including being a major modulator of hepatic inflammation and fibrosis in viral and non-viral liver diseases, and modulating HCC risk, metabolic phenotypes and other viral infections (CMV, EBV, HSV and HTLV-1). Thus, the significance of the original discovery has only continued to grow with time.

Alcoholic liver disease as a systemic disease

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Excessive ethanol consumption affects virtually any organ, both by direct and indirect mechanisms. According to a traditional liver-centered view, liver has been considered as the main victim of the harmful use of alcohol. However, many animal and human studies during the last two decades have widened the knowledge about alcoholic liver disease (ALD) pathophysiology, suggesting ALD have to be considered a true systemic disease. In addition to the classic consequences of endotoxemia associated with liver cirrhosis that were described several decades ago, growing evidence have shown that cytokines may also induce damage to the digestive tract, the central and peripheral nervous systems, the heart and vascular system, the bone and skeletal muscle, the endocrine and immune systems and disruption of nutritional status and, finally, cancer. Inflammation is closely linked with development of ALD. Acute inflammation as a defense against noxious stimuli is important for homeostasis in the body, whereas chronic exposure to an agent that induces inflammation may cause a dysregulated or unresolved inflammatory response, which causes chronic inflammation. The major sources of chronic low-grade inflammation in ALD are categorized as follows: a direct inflammatory cascade from the alcohol detoxification process and an indirect inflammatory cascade in response to gut microflora-derived lipopolysaccharides (LPS). The cytokines derive primarily from activated Kupffer cells exposed to Gram-negative intestinal bacteria, which reach the liver in supra-physiological amounts due to ethanol-mediated increased gut permeability. And reactive oxygen species (ROS) that enhance the inflammatory response are generated both by activation of Kupffer cells and by the direct metabolic effects of ethanol. The effects of ethanol metabolism and increased cytokine and ROS production are by no means restricted to the liver. Cytokines and LPS may induce damage in remote organs, even in those without significant liver disease. There is a clear-cut relation between oxidative damage and inflammation and alcoholism-associated diseases such as brain dysfunction, bone and muscle diseases, lung alterations, increased severity of infections, malnutrition, and an increased prevalence of cardiovascular disease or cancer. In conclusion, ALD should not be considered limited to the liver but as a true systemic disease and alcohol-related diseases can be viewed as an inflammatory disease, a concept which opens the possibility of using new therapeutic weapons to treat some of the complications of this devastating and frequent disease.

Management of NASH

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Over the last three decades, nonalcoholic fatty liver disease (NAFLD) has risen to become the most prominent cause of chronic liver disease in most affluent countries. Despite the rising prevalence, approved pharmacotherapies that alter long term clinical outcomes are still not available. Therapies for NAFLD/NASH can broadly be divided into those that target liver-related outcomes, and those that treat its extra-hepatic manifestations. In this regard, it is crucial for physicians to understand that more than 90 % of mortality from NAFLD relates to its extra-hepatic manifestations including cardiovascular disease, cancer and diabetes related mortality. Hence, it crucial that treatment should focus on the modulation of risk factors for cardiovascular disease and diabetes progression, namely the treatment of hypertension, dyslipidemia, smoking, and control of concomitant diabetes. The hepatic consequences of NAFLD include adverse outcomes related to end stage liver disease and liver failure, as well as hepatocellular cancer. Level 1 evidence for pharmacological treatment strategies are lacking but a variety of approaches have been shown to reduce histological disease progression. No study however has been of sufficient quality or duration to demonstrate improved liver-related outcomes. Several reports demonstrate clear evidence for lifestyle intervention in improving the histological features of inflammation and fibrosis. These improvements must be interpreted in the context of clear and unequivocal evidence that lifestyle intervention reduces progression to diabetes and improves long term clinical outcomes. Pharmacotherapies are a hot area for therapeutic development in fatty liver disease. Metformin by and large has not been shown to significantly improve liver histology, while, vitamin E and PPAR agonists improve steatohepatitis, with some improvement in fibrosis. However, potential adverse effects has meant that these agents are not uniformly or widely used. Omega three fatty acids had a sound theoretical basis for liver fat reduction, but a large study has shown that it did not reduce steatohepatitis. More recently, an array of pharmacological agents have been tested in phase 2 and earlier trials. These include obeticholic acid, liraglutide, a GLP-1 agonist, monoclonal antibodies against lysyl oxidase 2, and GFT505, a dual PPAR alpha delta agonist among others. Many of these are promising, especially for histological improvements, but await the completion of further therapeutic development and studies of long term outcomes. Finally, bariatric surgery for obesity does improve histological features in NASH, however level 1 evidence is required before it can be recommended for NASH as the sole indication.

NASH in Europe and Asia

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Non-alcoholic fatty liver disease (NAFLD) is a spectrum of liver disease that ranges from simple steatosis of the liver to progressive inflammation and fibrosis, resulting in non-alcoholic steatosis (NASH) and cirrhosis. The prevalence of NAFLD in the general population is about 3–50 %. In 14 European countries, prevalence of NAFLD is 33 % in adults. A study from Greece found that in 40 % of autopsied cases of ischaemic heart disease or traffic accident death have NASH. Prevalence of NAFLD in east part of the world is about 10–20 %. A study from Korea showed that from 589 potential liver

donors that underwent liver biopsy, about 51 % of NAFLD were observed. Another study from India showed that 16 % fatty liver were found from 1230 autopsies. From the previous studies, it has been established that the link between obesity and NAFLD increase the risk of NASH-related cirrhosis. Accumulated fat caused by excessive fat consumption, genetic factor, and other diseases result in liver disturbance to export triglyceride that have been formed. It cause the accumulation of tryglyceride in liver parenchym (hepatosteatosis). Inflammation that occurs due to obesity often manifest the presence of insulin resistant and produces inflammatory cytokines by adipose tissue or fat called adipokines. Asian population still maintain significant risks of metabolic syndrome and NAFLD, resulting primarily higher adiposity and visceral fat distribution. Furthermore, it may contribute to increased risk of NASH development. A study from Lesmana CR in 2009 showed that most of the metabolic syndrome features were found in patients with NASH. In this study, more than 80 % of patients were overweight or obese, 66.7 % were hyperlipidemia, and 16.7 % were type 2 diabetes mellitus. NAFLD is highly endemic in Europe where it represents a major potential threat to public health. More than 50 % of adults in 27 European countries are considered to be overweight or obese. Thus, the risk of NASH will also increasing in European countries as well.

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Symposium: Barriers of HCV treatment in Asia Pacific

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The Asian Pacific region has the biggest number of patients, three quarters of the world, suffering from liver diseases including Chronic Hepatitis, Cirrhosis and Hepatocellular Carcinoma due to HBV (Hepatitis B Virus) and HCV (Hepatitis C Virus) infections. Many of Asian countries have many patients suffering from B-viral related disorders, whereas a country like Japan has a little different demographic and clinical features, 70–80 % of patients with hepatocellular carcinoma are due to the C-viral related. Recent and remarkable progress of the development of oral drugs has made possible to eradicate HCV infection from all of my patients. In my clinic, I treated more than 350 patients in 6 months and in few HCV still persist. In this symposium, I will address the issues on HCV treatment in Asia, based upon my experience for the last 45 years.

New therapies in HCV: towards a global cure

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Hepatitis C has seen remarkable advances in therapy through clinical trials of all oral DAA therapy. Real life cure rates are in excess of 95 % similar to that reported from clinical trials. There are very few host demographic or viral factors that effect response although the presence of cirrhosis, degree of decompensation, African American race and genotype 3, have all shown some reduction in SVR. In this presentation we will discuss clinical trial and real life results and discuss specific issues such as baseline resistance testing, treatment failures, unmet needs and new evolving therapies. Finally, we will discuss the issues of global access and how to make the reality of conquering HCV possible on a global basis.

Unmet need for the patients with chronic hepatitis C

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Therapy for chronic hepatitis C virus (HCV) infection has been revolutionized due to interferon free all oral combinations with direct acting antiviral agents (DAA) targeting the HCV protease, polymerase and NS 5A protein. Sustained virological response (SVR) rates of more than 90 % for most HCV patients have become a reality and these results have been confirmed in real life experiences. The average treatment duration is 12 weeks, for some difficult to treat patients 24 weeks seem necessary while 8 weeks are sufficient for treatment naïve non cirrhotic HCV genotype 1 patients with viral load <6 MIO IU/ml with sofosbuvir (SOF) plus ledipasvir (LDV). Even shorter durations are presently explored for future regimen. Preliminary results suggest that 6 weeks may become possible at least for easy to treat patients. While this seems to be the limit for chronic HCV patients, 4 weeks seem realistic for acute HCV. HCV genotype 3 patients are now the most difficult to treat population. In particular for cirrhotic and treatment experienced patients effective and safe 12 week regimen are still needed. Pretreatment routine testing for resistance associated variants (RAV) seems not to be necessary for the average HCV patient. However, preexistent NS 5A mutants indicate a significant risk for treatment failure and they persist long term after treatment cessation. The relevance of viral resistance has to be studied further in DAA failure patients which is a very heterogeneous group. With the advent of DAA therapies prevention of recurrent HCV infection after liver transplantation has become possible. If patients achieve SVR before transplantation or are at least 30 days negative while under therapy HCV recurrence is prevented. Eradication of HCV infection in the posttransplant population should be achieved soon in the years to come and a decline of transplantations for HCV induced liver disease should happen within the next decade. SOF based DAA therapies have also been explored in decompensated liver disease, i.e. Child B and C cirrhosis. The majority of patients improve their MELD and CPT scores after achieving SVR. However, there is a proportion of patients that does not improve or even deteriorates. The definition of the point of no return is a major unmet need and still an unsolved problem. Renal insufficiency is another yet unsolved problem in particular in the decompensated patient since SOF based regimen cannot be given to patients with GFR <30 ml/min. On the other hand the so-called 3DAA combination (Paritasvir, Ombitasvir, Dasabuvir) can be given to patients with renal insufficiency but is either not recommended or contraindicated in decompensated liver disease. Novel regimen are needed for patients with decompensated liver disease and renal insufficiency. Whether SVR following successful DAA therapies protects from de novo infections at least in a proportion of patients also needs to be clarified. The biggest unmet need as of today is the global access to HCV therapies. A global strategy for HCV eradication is urgently needed.

Clinical outcomes of anti-hepatitis C therapy: the added benefits of all oral treatment

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Following marketing of the nucleotide polymerase inhibitor Sofosbuvir (SOF Gilead Sciences), the most awaited, golden era of interferon-free therapy of chronic hepatitis C, has begun. Such a remarkable shift in the therapeutic paradigm of hepatitis C has been consolidated by the publication on the row of a series of multinational studies with oral regimens, one combining Sofosbuvir with Ribavirin (RBV) or the inhibitor of the NS5A replication complex Ledipasvir (LDV) or Daclatasvir (DCV BMS), or the second wave inhibitor of NS3/4A protease, Simeprevir (SMV Janssen), while there is another regimen based on multiple direct acting antiviral agents (3D Abbvie) including an inhibitor of NS3/4 protease and the NS5A replication complex and a non nucleoside inhibitor of NS5B polymerase of HCV. Remarkably, a 12 week course with any regimen combining more than one DAA led to permanent sterilization of more than 90 % of the difficult to cure patients infected by genotype 1 or 4 HCV independently on virus subtype or disease severity, only requiring extended treatment to 24 week and or combination with RBV in patients with cirrhosis and a previous failure to interferon or with immune defect due to liver transplantation. The same holds true for SOF + RBV in genotype 2 whereas the currently best options for genotype 3 are the combinations of SOF with either LDV or DCV. Compared to interferon based therapies all orals are expected to provide additional survival benefits through an expanded access to treatment and eradication of HCV in patients with decompensated liver disease and organ transplants, who are interferon unable. There are, however, differences between regimens: 3D regimen is less effective in genotype 1a than in 1b, is unsafe in the treatment of patients with decompensated cirrhosis and is difficult to manage in liver transplanted patients and patients taking multiple medicines. In the last meeting of the European Association for the Study of the Liver (EASL, Vienna April 2015), the show was stolen by the reports of equally effective oral regimens composed by different class of antivirals administered for 6–8 weeks only, a strategy endorsed by big Pharma aiming at reducing the costs of antiHCV therapy while granting broad access and response to therapy. Yet, all oral therapy still needs refinement, as shown by the suboptimal rates of success in treatment-experienced cirrhotics infected by genotype 3 of HCV, severely decompensated (Child-Pugh C) patients with any genotype and patients with renal failure. The recognition that virtually all failures in difficult to cure patients materialize as a relapse after a successful end-of-therapy response and are driven by accumulation of resistant virions (particularly NS5A variants), calls for refining treatment strategies in terms of duration and use of RBV in patients at risk of failure. Finally, the fact that such a rapid and to some extent dramatic shift of the therapeutic paradigm of hepatitis C comes at the expenses of harsh arguments in the field of distributive justice being strongly constrained by the burden of costs, has widely solicited treatment strategies driven by prioritization criteria.

Aggressive surgical treatment of intrahepatic cholangiocarcinoma

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The incidence of intrahepatic cholangiocarcinoma (ICC) has been reported to increase for the last few decade. The surgical treatment is still a mainstay of treatment for ICC. However the surgical resection rate of ICC is not always high due to its diagnosis at the late stages. The advanced ICC usually involves the surrounding vasculatures such as the portal vein, hepatic artery, hepatic vein, and inferior vena cava, and also involves the adjacent organs such as the bile duct, gastroduodenum, colon, and pancreas. Therefore, aggressive surgical procedures with combined vascular resection and adjacent organ resection. In our series at Chiba University Hospital, we have resected 115 patients of ICC between. Major hepatectomy greater than hemi-hepatectomy were done in 84 patients (73 %). Combined vascular resection were done in 34 patients (30 %), the portal vein in 11, IVC in 15 and PV + IVC in 8 patients. Combined bile duct resection were done in 64 (58 %). Systematic lymph nodes dissections were done in 97 (84 %). The multivariate analysis of prognostic factors for overall survival after R0 surgical resection revealed a preoperative serum CA19-9 level as a only independent prognostic factor among tumor characteristics, 5-year survival rate; 30.3 and 0 %, in the group of <1000 and >1000, respectively ($p < 0.001$). Lymph node metastases was not a significant factor, when R0 resection was achieved, 5-yr survival rate, 27.7 and 11.8 % in the group of n0 and n1. The most frequent sites of metastatic lymph nodes of ICC was hilar nodes in 73 and 72 % of right-sided ICC and left-sided ICC, respectively. The gastro-hepatic lymph nodes were also important as a first metastatic lymph node station in left-sided ICC, 0 and 31 % of right-sided ICC and left-sided ICC, respectively. The group of combined vascular resection had similar long-term survival as the non-vascular resection group, 16 and 19 % of 5-year survival rate, respectively ($p = 0.36$). For the patients with initially unresectable locally advanced ICC, down-sizing chemotherapy has been administered and could brought about favorable anti-cancer effects to render resectable in 20–30 % of the patients. The survival after surgical resection following neo-adjuvant down-sizing chemotherapy was significantly better than that of the chemotherapy alone group. However, most patients with ICC had recurrence even after R0 resection. For the recurrence of ICC after surgical resection, repeat surgical resections were done in 15 patients, and achieved 25 % of 5-year survival rate which was significantly better than that of chemotherapy alone and best supportive care groups ($p < 0.0001$). Surgical resection could bring about the most favorable outcome of the prognosis in comparison with other treatment modalities in the patients with ICC. Therefore, HPB surgeon should surgically resect advanced ICC by using combined vascular resection and/or neo-adjuvant down-sizing chemotherapy even for initially unresectable and locally recurrent ICC.

Treatment of advanced HCC in BCLC B/C

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Patients with compensated cirrhosis, a hepatocellular carcinoma (HCC) stage beyond the Milan criteria and neither macroscopic vascular invasion by tumour cells nor symptoms of neoplastic disease (stage BCLC-B) are rarely considered for radical treatment. In those patients, repeat treatment with transarterial chemoembolization (TACE) was associated with improved survival of an average of 16–20 months, thereby qualifying TACE as a standard of care in BCLC-B patients, however with the recommendation to restrict

treatment to patients with Child–Pugh A or B cirrhosis, with or without small oesophageal varices to minimize the risk of hepatic decompensation that may follow procedure-induced liver ischaemia. Nevertheless, having survival benefits following TACE been observed in patients with a 3-year predicted spontaneous survival between 8 and 23 % (significantly worse than the 50 % predicted spontaneous survival of BCLC-B patients) (Llovet et al. 1999), TACE has been used to treat advanced (BCLC-C) patients, too. At this point, a redefinition of selection criteria and endpoints in TACE studies is deemed necessary, considering also that TACE with doxorubicin-loaded beads can cause complete necrosis in less than 5 cm tumors, standing therefore as a potentially radical treatment in selected patients. Further, there are reports of clinical benefits of radioembolization with yttrium 90 in this patient population. In BCLC-C patients, HCC is complicated by radiological evidence of portal vein thrombosis or extrahepatic tumour invasion as well as symptoms of neoplasia (performance status 1 or 2). In these patients, whose spontaneous survival does not exceed an average of 6 months, the multi-kinase inhibitor sorafenib is the standard of care. In Italy, sorafenib therapy is restricted to BCLC-C patients with compensated liver disease (Child–Pugh A) who have either a clinical or a radiological response during the first 2 months of therapy. In the last years, RECIST radiological criteria have been replaced by the modified criteria (mRECIST) that include early modifications of arterial vascularization of the tumour caused by sorafenib and better identify patients who respond to sorafenib. Optimization of sorafenib therapy may be obtained through down-dosing in patients with severe adverse events or poor tolerability, while it is possible to spare unnecessary morbidity through early identification of non responders based on the pattern of tumor progression during sorafenib administration. All targeted therapies competing with sorafenib as a first line treatment of HCC, have so far failed to demonstrate either non inferiority or superiority with respect to sorafenib. Currently, there is no validated second line treatment option for non responders to sorafenib.

Generation of Functional human liver from pluripotent stem cell

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Organ transplantation is the only curative method for treating end-stage organ failure. Over the past decade, there has been increased demand for organ transplantation throughout the world owing to the increased incidence of organ failure. To address such important clinical issue, a critical shortage of donor organs highlights the urgent need for generating organs from human pluripotent stem cells. Here we show the generation of functional human organ from human induced pluripotent stem cells (iPSCs) by transplantation of organ buds created in vitro. Human vasculatures in iPSC-organ bud transplants became functional by connecting to the recipient's vessels. The formation of functional vasculatures stimulated the maturation of iPSC-organ bud into tissue resembling the adult organ. This method is the alternative way to the generation of functional human organ from pluripotent stem cells. Although efforts must ensue to translate these techniques to treatments for patients, this proof-of- concept demonstration of organ-bud transplantation provides a promising new approach to a critical shortage of donor organs for treating end-stage organ failure.

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