Adv Ther (2023) 40:1171–1186
https://doi.org/10.1007/s12325-022-02416-7

ORIGINAL RESEARCH

Complement C3 Facilitates Stratification of Stages of Chronic Hepatitis B and Signifies Development of Acute-on-Chronic Liver Failure in Acute Decompensated Cirrhosis

Chong Chen · Zhu Yuan · Weixia Li · Ling Fei · Liujuan Ji · Qin Huang · Shuye Zhang · Liang Chen

Received: November 16, 2022 / Accepted: December 19, 2022 / Published online: January 18, 2023

ABSTRACT

Introduction: Patients with chronic hepatitis B (CHB) have a dynamic disease process and risk of end-stage liver disease. It is critical to unambiguously differentiate the stages of the disease and focus on therapy prior to onset of an irreversible clinical endpoint.

Methods: We retrospectively analyzed a wide range of CHB patients at different stages. The predictive power of serum complement component 3 (C3) levels for the development of acute-on-chronic liver failure (ACLF) in patients with decompensated cirrhosis was established and validated.

Results: The decrease in serum C3 levels paralleled the severity of diseases related to hepatitis B virus. Patients with decompensated cirrhosis who developed ACLF had significantly lower serum C3 levels than others on admission (0.50 vs. 0.80 g/L, $P < 0.001$). Data analysis also revealed that low serum C3 was a significant risk factor for developing ACLF (hazard ratio = 0.32, $P < 0.01$). The area under the receiver operating characteristic curve (auROC) for serum C3 levels that predicted the development of ACLF in patients with decompensated cirrhosis was 0.90, which had sensitivity and specificity of 88.2% and 88.7%, respectively. A similar result was observed in the validation set (auROC = 0.86

Chong Chen and Zhu Yuan are co-first authors.

Liang Chen and Shuye Zhang are joint corresponding authors.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s12325-022-02416-7.

C. Chen · W. Li · Q. Huang
Department of Infectious Diseases, Shanghai Public Health Clinical Center, Fudan University, Shanghai 201508, China

Z. Yuan
Department of Endocrinology, Jinshan Branch of Shanghai Sixth People's Hospital, Shanghai 201599, China

L. Fei · L. Chen (✉)
Department of Hepatology, Shanghai Public Health Clinical Center, Fudan University, 2901 Caolang Road, Jin-Shan District, Shanghai 201508, China
e-mail: chenliang@shphc.org.cn

L. Ji
Department of Severe Liver Disease, Shanghai Public Health Clinical Center, Fudan University, Shanghai 201508, China

S. Zhang (✉)
Clinical Center for BioTherapy, Zhongshan Hospital, Fudan University, No.180 Fengling Road, Xuhui District, Shanghai 200031, China
e-mail: shuye_zhang@fudan.edu.cn

for predicting development of ACLF in patients with decompensated cirrhosis).
Conclusions: Serum C3 levels are valuable in assessing the severity of CHB-related stages. Low C3 levels signifies the development of ACLF in patients with decompensated cirrhosis.

PLAIN LANGUAGE SUMMARY

Generally, acute-on-chronic liver failure is a rapidly worsening liver failure syndrome. This disease is intractable and with high mortality. Acute decompensation of the liver is defined as the occurrence of complications of liver disease (i.e., ascites, hepatic encephalopathy, gastrointestinal bleeding, and bacterial infection). Clinically, acute decompensation in hepatitis B virus-related cirrhosis (a result of chronic liver injury by virus) often develops into acute-on-chronic liver failure. In addition, the complement component 3 is a serum protein, which participates in the immune response against virus infection and has been reported to be associated with liver failure. We tried to explore the feature of serum complement component 3 to differentiate the stages of the disease and assess its predictive value for acute-on-chronic liver failure. So, we analyzed the complement component 3 data from a broad range of hepatitis B virus-cirrhosis patients. Through analysis, we found that complement component 3 levels are valuable in assessing the severity of chronic hepatitis B-related stages. Low complement component 3 levels can also signify the development of acute-on-chronic liver failure in patients with decompensated cirrhosis.

Keywords: Complement C3; Hepatitis B virus; Cirrhosis; Acute-on-chronic liver failure

Key Summary Points

Why carry out this study?

Chronic hepatitis B (CHB) has a dynamic disease process and acute decompensation (AD) on hepatitis B virus (HBV)-related cirrhosis often develops into acute-on-chronic liver failure (ACLF).

At present, little is known about warning biomarkers for the occurrence and progression of ACLF among HBV cirrhosis patients with AD.

What was learned from the study?

The decrease in serum complement component 3 (C3) levels paralleled the severity of diseases related to HBV ($P < 0.05$). The area under the receiver operating characteristic curve (auROC) for serum C3 levels that predicted the development of ACLF in patients with decompensated cirrhosis was 0.90, which had sensitivity and specificity of 88.2% and 88.7%, respectively. A similar result was observed in the validation set (auROC = 0.86).

Serum C3 levels are valuable in assessing the severity of CHB-related stages. Low C3 levels signifies the development of ACLF in patients with decompensated cirrhosis.

INTRODUCTION

Hepatitis B virus (HBV) infection is a common health problem worldwide. Several challenges remain regarding the elimination of HBV infection; in particular, the lack of a definitive cure for HBV carriers [1, 2]. HBV-related liver cirrhosis manifested as a structural disorder and liver dysfunction, with no cure. Although HBV-related compensated cirrhosis may not have clear clinical manifestations, it does present a high risk of end-stage liver disease, including

acute decompensation (AD) and acute-on-chronic liver failure (ACLF). ACLF manifests is related to dysfunction and failure of major organs, with rapid disease progression and high short-term mortality [3–5]. Moreover, AD in HBV-related cirrhosis often develops into ACLF. The intractability and high mortality of ACLF mean that it is critical to unambiguously differentiate the stages of the disease and focus on therapy prior to the onset of an irreversible clinical endpoint. Nevertheless, at present, little is known about warning biomarkers for the occurrence and progression of ACLF among HBV cirrhosis patients with AD.

The complement system participates in the immune response against infection [6, 7]. Activated complement component 3 (C3) can contribute to extensive tissue damage by increasing the production of proinflammatory cytokines from T lymphocytes [8]. Deposition of C3 in infected tissue can be observed after various viral infections, including influenza, coronavirus infection and hepatitis [7, 9, 10]. Reduced serum C3 levels has been observed in patients with liver failure [11]. More importantly, a limited number of studies have indicated that low levels of serum C3 suggest progression of HBV-ACLF and lead to a worse short-term prognosis [12–14]. However, the data regarding C3 are still scarce and incomplete. In this study, we analyzed the C3 data from a broad range of HBV-cirrhosis patients, with or without AD, and with ACLF, to capture the concise C3 signatures and evaluate their value in the diagnosis and prognosis of AD and ACLF related to HBV cirrhosis.

METHODS

Study Design and Cohorts

We screened patients with liver injury according to medical history and biochemical data from the Department of Hepatology and Department of Severe Liver Disease, Shanghai Public Health Clinical Centre, Fudan University, Shanghai, China (January 2020 to December 2021). A total of 1039 patients with chronic hepatitis B (CHB) were initially screened. CHB was diagnosed according to hepatitis B surface antigen and HBV DNA positivity over the previous 6 months according to the medical records [3]. The following patients were excluded: (1) pregnant women; (2) coexistence of other viral infection markers, hepatitis A, C, D or E virus; Epstein–Barr virus or cytomegalovirus; (3) severe underlying comorbidities (e.g. malignancy, or metabolic or immune disorders); (4) other types of chronic hepatitis: autoimmune/drug/alcoholic/fatty hepatitis; and (5) duplicate cases and patients who lost clinical data. Additionally, stable decompensated cirrhosis patients (characterized by chronic ascites or recurrent peritonitis) and non-first occurrence of hepatic encephalopathy, or gastrointestinal bleeding were also excluded by initial screening. Finally, 607 patients with CHB were assigned to different analysis groups according to whether they had underlying cirrhosis, AD or ACLF. There were 147 patients with CHB, 118 without AD of cirrhosis, 119 with AD of cirrhosis, and 223 with ACLF (Fig. 1).

The latter validation cohort was also screened and enrolled between January and June 2022 from the Department of Hepatology and Department of Severe Liver Disease, Shanghai Public Health Clinical Centre, Fudan University, Shanghai, China. There were 86 patients with AD of cirrhosis in the validation cohort. The patients with AD of cirrhosis and those with ACLF of cirrhosis were named HBV-C-AD and HBV-C-ACLF, respectively. The study was reviewed and approved for publication by the Institutional Review Board of the Shanghai Public Health Clinical Centre (NO.2022-S075-01). The requirement for individual written informed consent was waived since the study was retrospective in design, all patient information was anonymous and only currently existing data was used. We complied with the Helsinki Declaration 1964, and its later amendments.

Definition of Diseases and Outcomes

HBV infection was diagnosed according to hepatitis B surface antigen and HBV DNA positivity over the previous 6 months [3]. HBV-

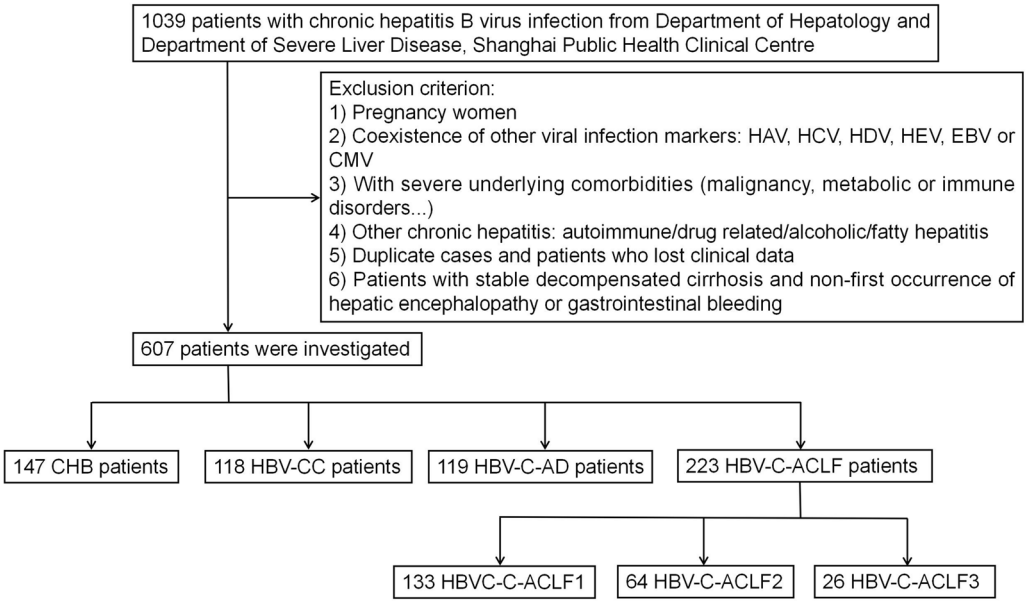

Fig. 1 Flow diagram of study participants. We enrolled 607 CHB patients in the discovery cohort. The groups of patients with AD of cirrhosis and patients with ACLF of cirrhosis were named HBV-C-AD and HBV-C-ACLF, respectively. *HAV* hepatitis A virus, *HCV* hepatitis C virus, *HDV* hepatitis D virus HEV, hepatitis E virus, *EBV* Epstein–Barr virus, *CMV* cytomegalovirus, *CHB* chronic hepatitis B, *HBV* hepatitis B virus, *HBV-CC*, hepatitis B virus-compensated cirrhosis, *AD* acute decompensation, *ACLF* acute-on-chronic liver failure

cirrhosis was defined histologically as distortion of the hepatic architecture and regenerative nodules, which was determined by liver biopsy or imaging (ultrasound, computed tomography, or magnetic resonance) [15]. Cirrhosis with no history of AD was defined as HBV-compensated cirrhosis (HBV-CC). AD was defined by acute development of one or more related complications of liver disease (i.e., ascites, hepatic encephalopathy, gastrointestinal bleeding, and bacterial infection) [4]. Patients with cirrhosis who developed a first episode of AD at the time of hospital admission or within 2 weeks before admission were assigned to the HBV-C-AD group. Patients with stable decompensated cirrhosis (characterized by chronic ascites or recurrent peritonitis) and non-first occurrence of hepatic encephalopathy, or gastrointestinal bleeding were excluded by initial screening. HBV-C-ACLF patients were identified according to the criteria of the Chinese Group on the Study of Severe Hepatitis B (COSSH): acute hepatic insult with severe jaundice (total bilirubin $\geq$ 12 mg/dL) and coagulopathy (INR $\geq$ 1.5) [16]. ACLF grades were also identified and stratified according to the COSSH criteria (Supplemental Materials). We followed the HBV-C-AD patients to establish whether they developed ACLF within 28 days after admission. The poor prognosis of patients was defined as mortality or liver transplantation (LT) within 28 days after admission. C3 levels were subject to receiver operating characteristic (ROC) curve analysis to evaluate their sensitivity and specificity to predict 28 days endpoints.

Assessment of Clinical Parameters

The patients underwent clinical evaluation and laboratory examination, and we obtained this information from the hospital clinical database. Clinical characteristics included age, gender, liver complications, and clinical outcomes. All parameters were measured in the hospital's clinical laboratory using standard techniques, including serum HBV markers, HBV DNA levels, routine blood tests, coagulation function tests, liver and renal function tests, and C3 levels.

Table 1 Baseline characteristics, liver complications and laboratory parameters of enrollment patients

Parameters	Total patients ($n = 607$)	CHB ($n = 147$)	HBV-CC ($n = 118$)	HBV-C-AD ($n = 119$)	HBV-C-ACLF ($n = 223$)	*P* value
Age (years)	40 (41–60)	40 (31–48)	53 (45–59)	59 (48–71)	52 (45–62)	< 0.001
Gender (male, %)	422 (69.5)	106 (72.1)	80 (67.8)	66 (55.5)	170 (76.2)	< 0.01
HBe Ag (+), *n* (%)	221 (36.4)	59 (40.1)	54 (45.8)	45 (37.8)	63 (28.3)	< 0.01
HBV DNA, (IU/mL)						
< 500	175 (28.8)	19 (12.9)	59 (50.0)	43 (36.1)	54 (24.2)	< 0.001
500–10^5	251 (41.4)	56 (38.1)	41 (34.7)	46 (38.7)	108 (48.4)	
> 10^5	181 (29.8)	72 (49.0)	18 (15.3)	30 (25.2)	61 (27.4)	
Clinical complications at enrollment, *n* (%)						
Ascites	255 (42.0)	–	–	84 (70.6)	171 (76.7)	0.14
Bacterial infection	93 (15.3)	–	–	25 (21.8)	68 (30.5)	< 0.01
Hepatic encephalopathy	127 (20.9)	–	–	30 (25.2)	97 (43.5)	< 0.01
Gastrointestinal hemorrhage	11 (1.8)	–	–	4 (3.4)	7 (3.1)	0.57
Laboratory parameters						
ALT, U/L	199 (54–551)	521 (66–1268)	88 (25–271)	102 (33–211)	139 (48–473)	< 0.001
AST, U/L	159 (39–433)	357 (42–762)	77 (16–168)	89 (25–177)	120 (62–286)	< 0.001
Tbil, mg/dL	8.23 (5.23–10.71)	8.20 (5.41–9.44)	4.68 ± 1.65	3.72 ± 2.20	21.2 (11.5–29.6)	< 0.001
Albumin, g/L	33.6 (31.9–37.1)	35.2 (33.6–38.9)	32.8 (29.0–37.5)	30.9 (27.6–35.8)	31.4 (28.2–35.0)	< 0.05
INR	1.39 (1.21–1.78)	1.13 (0.80–1.25)	1.20 ± 0.87	1.42 (1.30 ± 1.62)	2.14 (1.63–2.95)	< 0.001
Creatinine, μmol/L	59.8 (47.0–70.0)	56.1 (48.6–67.2)	55.0 ± 12.3	60.1 (45.9–72.3)	63.9 (50.4–79.8)	< 0.05
Urea, mmol/L	4.57 (3.99–5.19)	2.73 (2.51–3.01)	2.88 (2.63–3.78)	3.10 (2.70–4.92)	5.09 (3.44–7.03)	< 0.05
Glucose, mmol/L	6.01 (4.76–8.16)	6.11 (5.11–8.03)	6.32 (5.53–8.41)	6.09 (4.91–8.07)	6.83 (5.03–8.56)	0.58
WBC, 10^9/L	5.70 (4.11–7.31)	5.28 (3.79–6.66)	5.81 (3.98–7.98)	5.61 (4.10–7.34)	6.08 (4.40–8.35)	0.67
Hemoglobin, g/L	110 (106–127)	115 (102–133)	118 (103–138)	109 ± 16	115 ± 22	0.34

Table 1 continued

Parameters	Total patients ($n = 607$)	CHB ($n = 147$)	HBV-CC ($n = 118$)	HBV-C-AD ($n = 119$)	HBV-C-ACLF ($n = 223$)	*P* value
Platelet, 10^9/L	166 (121–182)	189 ± 55	133 (91–196)	94 (62–139)	88 (51–135)	< 0.05
C3, g/L	0.73 (0.43–0.95)	0.99 ± 0.14	0.84 ± 0.18	0.72 ± 0.25	0.36 (0.25–0.55)	< 0.001

The patients with AD of cirrhosis and those with ACLF of cirrhosis were named HBV-C-AD and HBV-C-ACLF, respectively. Cirrhosis with no history of AD was defined as HBV-compensated cirrhosis (HBV-CC). Data are presented as the median (Q1-Q3) or the number of patients (%). The categorical data between two groups were compared with Chi-square test or Fisher's exact test. The continuous data between two groups were compared by *t* test or Mann–Whitney-test *CHB* chronic hepatitis B, *HBV-CC* hepatitis B virus-compensated cirrhosis, *AD* acute decompensation, *ACLF* acute-on-chronic liver failure, *ALT* alanine aminotransferase, *AST* aspartate aminotransferase, *Tbil* total bilirubin, *INR* international normalized ratio, *WBC* white blood cells, *C3* complement component 3

Calculation prediction score of HBV-ACLF was according to the COSSH criteria [16, 17]: COSSH-ACLFs = 0.741 × [international normalized ratio (INR)] + 0.523 × HBV SOFA + 0.026 × age + 0.003 × total bilirubin; COSSH ACLF IIs = 1.649 × ln(INR) + 0.457 × hepatic encephalopathy score (0, 1 point; grade ½, 2 points; grade 3/4, 3 points) + 0.425 × ln(neutrophil) + 0.396 × ln(total bilirubin) + 0.576 × ln(serum urea) + 0.033 × age.

Statistical Analysis

All statistical analysis in this study was performed using SPSS 28.0 software, MedCalc Software (MedCalc Sofware, Belgium) and GraphPad Prism (GraphPad Software). The Kolmogorov–Smirnov test was used to check the normality of data. Normal continuous variables were presented as the mean ± SD, non-normal variables were presented as the median and range (Q1–Q3), and categorical variables were presented as the number (%). The categorical data between two groups were compared with Chi-square test or Fisher's exact test. The continuous data between two groups were compared by *t* test or Mann–Whitney-test. A multivariate Cox regression model was used for examining the risk factors for developing ACLF and poor prognosis. Correlation of two variables were calculated by Spearman's correlation analysis. Areas under receiver operator characteristic (auROC) curves were built to assess the ability of C3 levels to predict progression and prognosis of disease. All significance tests were two-tailed, and $P < 0.05$ was considered as significant difference between groups.

RESULTS

Patient Characteristics and Comparison

The baseline clinical characteristics of the discovery cohort are summarized in Table 1. Patients in this study were middle-aged, with a median age of 40 years and 69.5% were male. We investigated the clinical data and differences between the CHB, HBV-CC, HBV-C-AD, and HBV-C-ACLF groups. There were significant differences among each group, including age, gender, hepatitis B e antigen positivity, HBV DNA levels, clinical complications and other laboratory parameters. There were no significant differences between glucose, white blood cell count and hemoglobin levels. The CHB group had the highest level of C3, the stable HBV-CC group had a moderate level of C3, the HBV-C-AD group had a low level of C3, and the HBV-C-ACLF group had the lowest level of C3 ($P < 0.001$) (Table 1; Fig. 2A). These data suggest that the variation in C3 level may be associated with stratification of the severity of HBV-induced diseases.

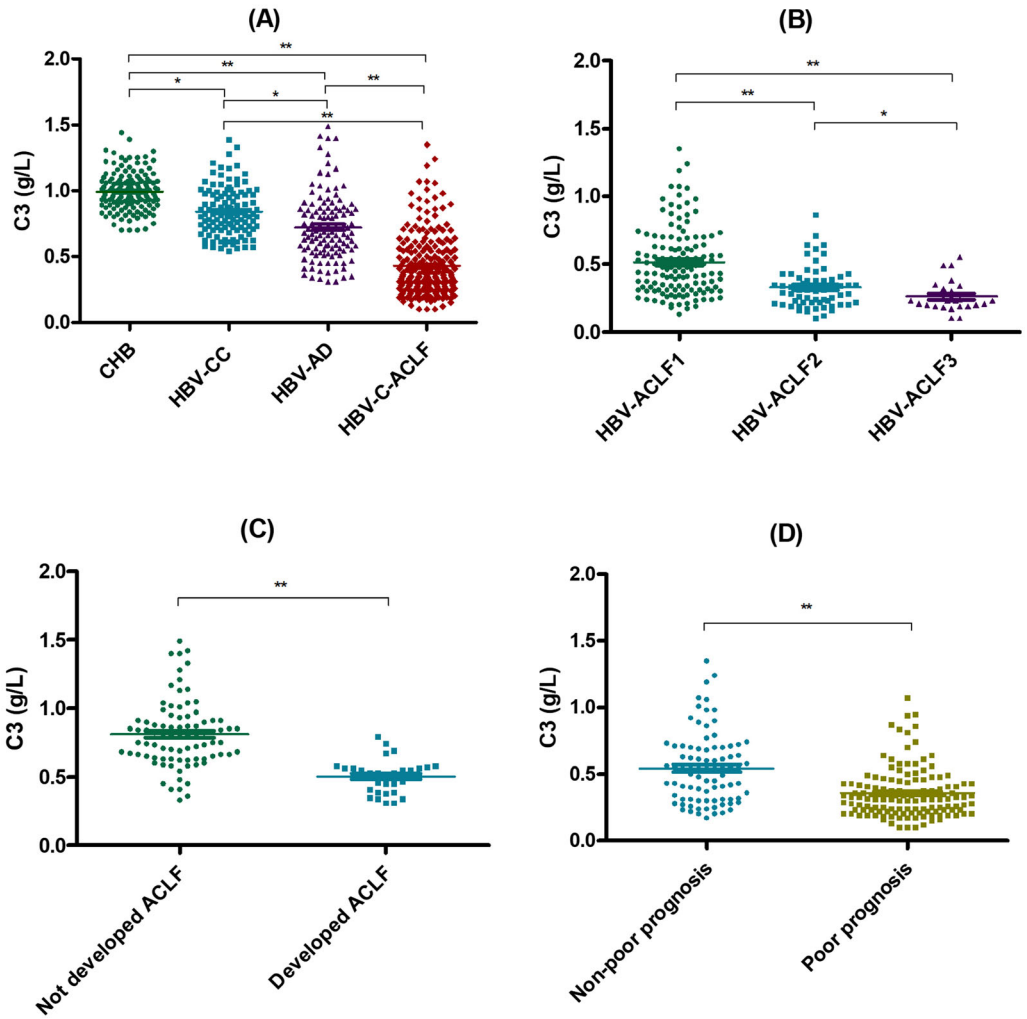

Fig. 2 C3 levels varied with disease severity, ACLF clinical grades, AD progression and clinical outcomes of ACLF. **A** C3 levels were associated with disease stratification in CHB. **B** C3 levels differences between ACLF clinical grades. **C** Patients with HBV-C-AD who developed ACLF had lower serum C3 levels than those did not develop ACLF. **D** Patients with HBV-C-ACLF with poor prognosis had lower serum C3 levels than those without poor prognosis. The patients with AD of cirrhosis and those with ACLF of cirrhosis were named HBV-C-AD and HBV-C-ACLF, respectively. Follow up the HBV-C-AD patients to establish whether they developed ACLF within 28 days after admission. ACLF grades were identified and stratified according to the COSSH criteria. The poor prognosis of patients was defined as mortality or liver transplantation within 28 days after admission. * < 0.05; ** < 0.01. *C3* complement component 3, *CHB* chronic hepatitis B, *HBV-CC* hepatitis B virus-compensated cirrhosis, *AD* acute decompensation, *ACLF* acute-on-chronic liver failure, *COSSH* Chinese group on the study of severe hepatitis B

C3 Levels Varied by AD Progression and ACLF Clinical Grades

We followed the clinical outcomes of HBV-C-AD patients within 28 days after admission. Patients were assigned to two groups based on whether they developed ACLF: group 1 ($n = 34$, 28.6%) developed ACLF and group 2 ($n = 85$, 71.4%) gradually recovered after treatment. Baseline C3 levels were compared between these two groups (Table 2 and Fig. 2C). The patients who developed ACLF had lower baseline serum

Table 2 The characteristics and risk factors analysis between patients developed and not developed ACLF in HBV-C-ADs

Parameters	Developed ACLF ($n = 34$)	Not developed ACLF ($n = 85$)	*P* value	HBV-C-ADs ($n = 119$)	Univariate analysis		Multivariate analysis	
					HR (95 CI%)	*P* value	HR (95 CI%)	*P* value
Age (years)	61 ± 14	59 (48–71)	0.381	59 (48–71)	NA	0.818		
Gender (male, %)	16 (47.1)	50 (58.8)	0.308	66 (55.5)	0.98 (0.72–1.07)	0.321		
HBe Ag (+), *n* (%)	15 (44.1)	30 (35.3)	0.407	45 (37.8)	1.01 (1.00–1.01)	0.462		
HBV DNA, (IU/mL)								
< 500	16 (47.1)	27 (31.8)	0.180	43 (36.1)	Reference	Reference		
500–10^5	9 (26.5)	37 (43.5)		46 (38.7)	0.76 (0.39–0.99)	0.063		
> 10^5	9 (26.5)	21 (24.7)		30 (25.2)	1.00 (0.99–1.01)	0.885		
Clinical complications at enrollment, *n* (%)								
Ascites	20 (58.8)	64 (75.3)	0.118	84 (70.6)	0.95 (0.91–1.08)	0.077		
Bacterial infection	11 (32.4)	14 (16.5)	0.080	25 (21.8)	2.89 (1.77–5.22)	< 0.01	2.01 (1.56–3.03)	< 0.05
Hepatic encephalopathy	12 (35.3)	18 (21.2)	0.160	30 (25.2)	1.89 (1.41–2.13)	< 0.05	1.50 (1.13–1.91)	< 0.05
Gastrointestinal hemorrhage	4 (11.7)	0	< 0.01	4 (3.4)	3.11 (2.10–3.82)	< 0.01	1.85 (0.99–2.76)	0.084
Laboratory parameters								
ALT, U/L	88 (26–201)	131 (49–254)	0.149	102 (33–211)	0.89 (0.51–1.11)	0.540		
AST, U/L	67 (19–166)	101 (33–205)	0.070	89 (25–177)	0.61 (0.39–1.18)	0.073		

Table 2 continued

Parameters	Developed ACLF ($n = 34$)	Not developed ACLF ($n = 85$)	*P* value	HBV-C-ADs ($n = 119$)	Univariate analysis		Multivariate analysis	
					HR (95 CI%)	*P* value	HR (95 CI%)	*P* value
Tbil, mg/dL	4.10 (2.10–7.01)	2.80 ± 1.60	< 0.05	3.72 ± 2.20	2.22 (1.52–3.25)	< 0.05	1.08 (0.99–1.26)	0.671
Albumin, g/L	31.0 ± 5.9	30.3 (26.2–34.8)	0.654	30.9 (27.6–35.8)	1.00 (0.99–1.00)	0.821		
INR	1.52 (1.38–1.70)	1.33 (1.12–1.42)	< 0.05	1.42 (1.30 ± 1.62)	1.89 (1.28–2.71)	< 0.05	1.11 (0.94–1.68)	0.447
Creatinine, μmol/L	67.1 ± 8.0	55.1 (41.0–69.8)	0.062	60.1 (45.9–72.3)	1.17 (0.42–3.47)	0.692		
Urea, mmol/L	4.22 (3.01–6.27)	2.92 (2.32–5.03)	< 0.05	3.10 (2.70–4.92)	1.69 (1.34–2.76)	< 0.05	1.21 (0.94–1.73)	0.221
Glucose, mmol/L	5.91 (4.78–7.98)	6.22 (5.03–8.49)	0.324	6.09 (4.91–8.07)	1.00 (1.00–1.02)	0.323		
WBC, 10^9/L	5.25 ± 1.10	6.11 (4.60–8.23)	0.419	5.61 (4.10–7.34)	0.92 (0.88–1.06)	0.532		
Hemoglobin, g/L	111 ± 13	108 (91–121)	0.822	109 ± 16	1.01 (1.00–1.01)	0.803		
Platelet, 10^9/L	84 (58–112)	103 (77–151)	< 0.05	94 (62–139)	0.78 (0.37–0.98)	< 0.05	0.90 (0.57–1.06)	0.622
C3, g/L	0.50 ± 0.12	0.80 (0.65–0.91)	< 0.001	0.72 ± 0.25	0.17 (0.10–0.41)	< 0.001	0.32 (0.16–0.53)	< 0.01
Prognostic score								
COSSH-ACLFs	6.1 ± 0.8	5.4 ± 0.8	< 0.001	5.8 (4.8–6.1)	1.88 (1.16–2.44)	< 0.05	1.06 (0.52–2.00)	0.333

Table 2 continued

Parameters	Developed ACLF ($n = 34$)	Not developed ACLF ($n = 85$)	*P* value	HBV-C-ADs ($n = 119$)	Univariate analysis		Multivariate analysis	
					HR (95 CI%)	*P* value	HR (95 CI%)	*P* value
COSSH ACLF IIs	5.7 (5.2–7.0)	5.1 (4.5–5.7)	< 0.001	5.2 (4.8–6.0)	2.30 (1.42–3.91)	< 0.01	1.84 (1.11–2.73)	< 0.05

Patients with cirrhosis who developed a first episode of AD at the time of hospital admission or within 2 weeks before admission were assigned to the HBV-C-AD group. The categorical data between two groups were compared with Chi-square test or Fisher's exact test. The continuous data between two groups were compared by *t* test or Mann–Whitney test. Univariate and multivariate Cox regression models were then used to assess the associations between various risk factors and different clinical outcomes, as indicated

ACLF acute-on-chronic liver failure, *AD* acute decompensation, *HR* Hazard Ratio, *ALT* alanine aminotransferase, *AST* aspartate aminotransferase, *Tbil* total bilirubin, *INR* international normalised ratio, *WBC* white blood cells, *C3* complement component 3, *COSSH* Chinese group on the study of severe hepatitis B, *NA* not available

C3 levels ($P < 0.001$). Lower serum C3 was a significant risk factor for developing ACLF [hazard ratio (HR) = 0.32, $P < 0.01$]. Patients with HBV-ACLF may have had different clinical symptoms and outcomes according to ACLF grade; therefore, we analyzed C3 levels in three subgroups of patients according to ACLF grade: subgroup 1 consisted of 133 patients with ACLF 1; subgroup 2 included 64 patients with ACLF 2; and subgroup 3 included 26 patients with ACLF 3 (Supplementary Table 1 and Fig. 2B). ACLF 3 had the lowest level of C3 (0.26 vs. 0.33 g/L compared with ACLF 2, and 0.26 vs. 0.53 g/L compared with ACLF 1) ($P < 0.001$). Lower levels of C3 were correlated with higher grades of ACLF, which indicated worse clinical outcomes. We grouped all 223 HBV-C-ACLF patients into two groups, with or without poor prognosis. C3 levels were significantly lower among patients with poor prognosis (median 0.33 vs. 0.54 g/L, $P < 0.001$) than in those without poor prognosis (Supplementary Table 2 and Fig. 2D). C3 levels were significantly higher than those observed at admission in the recovery group ($P < 0.001$). C3 levels showed a continued downward trend (not significant) among those with a poor prognosis (Supplementary Fig. 1D).

Association Between Hepatic Complications, ACLF Scores and C3 Levels

To further examine the prognostic value of C3 levels in severe liver diseases, we examined liver complications in HBV-C-ACLF patients. As a continuous variable, baseline C3 levels were negatively associated with liver complications in HBC-C-ACLF patients ($P < 0.05$) (Supplementary Fig. 1A). The data also revealed a significant negative correlation between C3 levels and ACLF scores in HBV-C-ACLF (COSSH ACLFs: $r = -0.5192$, $P < 0.001$ and COSSH ACLF IIs: $r = -0.4666$, $P < 0.001$) (Supplementary Fig. 1B and C).

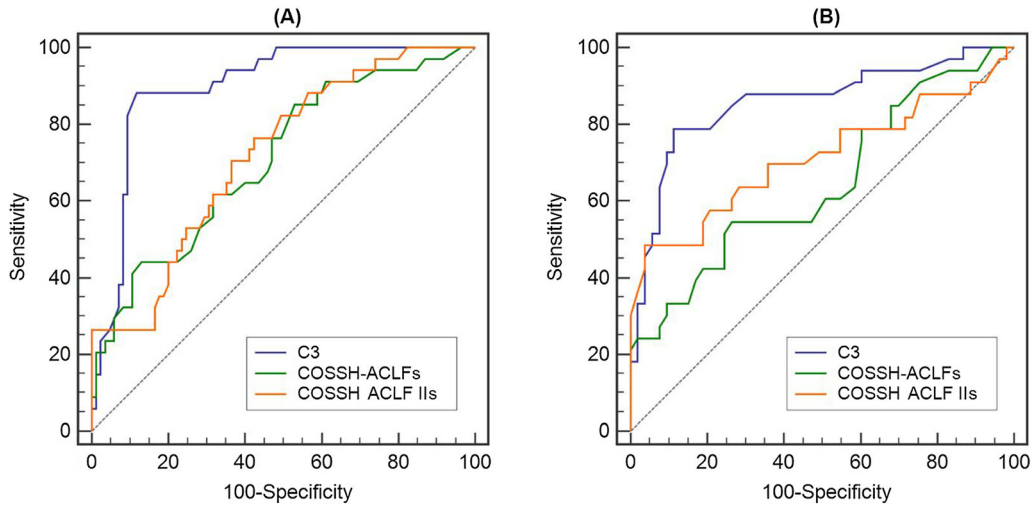

Fig. 3 ROC curves for predicting development of ACLF in 119 HBV-C-AD patients. **A** ROC curves for predicting development of ACLF in patients with AD of cirrhosis in the discovery cohort (auROC = 0.90, 0.71 and 0.72 for C3 levels, COSSH-ACLFs and COSSH ACLF IIs, respectively). **B** ROC curves for predicting development of ACLF in patients with AD of cirrhosis in the validation cohort (auROC of 0.86, 0.64 and 0.71 for C3 levels, COSSH-ACLFs and COSSH ACLF IIs, respectively). Follow up of the HBV-C-AD patients to establish whether they developed ACLF within 28 days after admission. AuROCs for different models were calculated and compared using the *Z* test (Delong's method). *C3* complement component 3, *COSSH* Chinese group on the study of severe hepatitis B, *auROC* the area under the receiver operating characteristic

Identification and Validation of the Predictive Power of Serum C3 Levels

There is a consensus that a critical intervention or therapeutic window may exist between the inducement and the development of ACLF to prevent the ACLF onset. We established that C3 levels correlated with disease progression. We then investigated whether C3 levels accurately predicted progression of AD in patients with HBV cirrhosis. ROC curve analysis was performed to predict the development of ACLF in HBV-C-AD patients. AuROC for serum C3 levels that predicted ACLF development within 28 days was 0.90, with sensitivity and specificity of 88.2% and 88.7%, respectively (Fig. 3A and Table 3). We used a validation cohort to test the predictive power of C3 levels for ACLF development. The baseline demographic and clinical data of the validation cohort were similar to those of the discovery cohort (Supplementary Table 3). A consistent result was found in the validation cohort, with an auROC of 0.86 (Fig. 3B and Table 3). Unlike the high predictive power of ACLF development, we found that C3 levels demonstrated lower predictive power for poor prognosis of ACLF (auROC = 0.73), while auROC for COSSH-ACLFs and COSSH ACLF IIs was 0.91 and 0.86, respectively) (Supplementary Fig. 2).

DISCUSSION

We studied the serum C3 levels in patients with HBV-related liver disease and analyzed its association with clinical features. A key finding was the variation of C3 levels as a function of disease severity. Data collected from a broad range of CHB patients with different severity showed a declining trend in C3 levels following disease progression, suggesting that the complement system is modulated by liver injury. CHB carriers may have a dynamic disease process, with intermittent exacerbation and unbalanced immune response [2, 18]. Dysregulated C3 levels imply that the complement system is activated, and varies during disease

Table 3 AuROCs of prognostic models for development of ACLF in 119 HBV-C-AD patients

Predictors	Development of ACLF in patients with decompensated cirrhosis									
	auROC	95 CI%	Cut-off	Youden	Sensitivity (%)	Specificity (%)	+ LP	−LP	PPV (%)	NPV (%)
Discovery cohort ($n = 119$)										
C3	0.90	0.84–0.96	5.8	0.76	88.2	88.7	7.50	0.13	75.0	94.9
COSSH-ACLFs	0.71	0.61–0.81	5.2	0.32	85.3	47.1	1.61	0.31	39.2	88.9
COSSH ACLF IIs	0.72	0.62–0.81	5.2	0.34	70.6	63.5	1.94	0.46	43.6	84.4
C3 vs. COSSH-ACLFs	$P = 0.0029$									
C3 vs. COSSH ACLF IIs	$P = 0.0018$									
Validation cohort ($n = 86$)										
C3	0.86	0.77–0.95	5.7	0.68	78.8	88.7	6.96	0.24	81.2	87.0
COSSH-ACLFs	0.64	0.52–0.77	5.8	0.28	54.6	73.4	2.06	0.62	56.2	72.2
COSSH ACLF IIs	0.71	0.59–0.84	6.4	0.45	48.5	96.2	12.90	0.54	88.9	75.0
C3 vs. COSSH-ACLFs	$P = 0.0034$									
C3 vs. COSSH ACLF IIs	$P = 0.0829$									

Patients with cirrhosis who developed a first episode of AD at the time of hospital admission or within 2 weeks before admission were assigned to the HBV-C-AD group. AuROCs for different models were calculated and compared using the Z test (Delong's method). The optimal cut-off points were determined by maximizing Youden index

AuROC the area under the receiver operating characteristic, *ACLF* acute-on-chronic liver failure, *AD* acute decompensation, *C3* complement component 3, *COSSH* Chinese group on the study of severe hepatitis B, $+LR$ positive likelihood ratio, $-LR$ negative likelihood ratio, *PPV* positive predictive value, *NPV* negative predictive value

development from CHB, stable cirrhosis and AD to ACLF.

The complement system is activated during hepatitis virus infection, whereby Kupffer cells in the liver are activated by C3 to mediate harmful inflammation, leading to severe liver damage [7, 19]. Earlier studies have suggested several explanations for the decreased serum complement levels from hepatotropic infection and related diseases [6, 12, 20, 21]. Firstly, the liver itself is the major site of small molecular protein synthesis, from which C3 is mainly produced. Hepatocytic and non-hepatocytic liver-resident immune cells (such as plasma cells, monocytes/macrophages and T lymphocytes) participate in the activation and regulation of complement. The major functions of the complement system are opsonization, cytolysis and phagocytosis, which are also closely linked with liver biology and pathology. Most raw materials and regulatory factors required for synthesis of complement are also mainly synthesized or expressed in hepatocytes. So, as liver injury worsens, liver synthetic processes are compromised and C3 levels are reduced. Secondly, antigen–antibody complexes can be

induced in the immune response following liver injury, which triggers activation of the complement system and excessive consumption of complement components. C3 can be cleaved into anaphylatoxin C3a and opsonin C3b following activation, of which C3a in turn can amplify immuno-inflammatory responses. The combination of the above processes results in significant decline in C3 levels as the disease progresses. As a result, reduced C3 levels are associated with poor liver function and refractory liver complications in these patients.

HBV-ACLF is a serious manifestation that commonly occurs following AD of cirrhosis. It is essential to implement aggressive treatment early during ACLF with the aid of sensitive and objective diagnostic measures [22, 23]. Usually, a number of scoring systems [model for end-stage liver disease (MELD), Child–Pugh and COSSH], biochemical indicators (liver enzymes, bilirubin or coagulation indicators) and clinical manifestations (ascites and hepatic encephalopathy) are used to monitor progression of HBV cirrhosis [16, 24, 25]. However, the above monitoring methods have some limitations, such as subjective judgment and limited diagnostic precision. Especially, it is unclear whether these predictive scoring systems are also valuable for the prognosis of HBV-C-AD. Therefore, extensive investigation is still needed to improve and refine diagnosis for early warning of ACLF development. It is important that the diagnostic value of other potential ACLF biomarkers, especially those involved in the pathophysiology of ACLF, should be considered [26, 27]. ACLF is believed to be caused by an excessive immune response against virus exacerbation or other acute insults, in which the complement system is closely involved [18, 23]. Previous studies [11, 13, 14] and data from this study have all found significant changes in C3 levels at all stages of HBV-related disease, but no study has yet examined its predictive value in end-stage cirrhosis. Here, we studied its predictive performance for ACLF in patients with AD. It is encouraging that our data showed that the predictive effect of C3 levels alone seemed to exceed the COSSH score in both the discovery and validation cohorts. Based on serum C3 levels, we can more accurately differentiate the probability of developing HBV-ACLF. In addition, we gained a better understanding of the relationship between the complement system and HBV-C-ACLF. Certainly, we also considered that the value of C3 as a marker should be validated in upcoming studies of more cohorts.

ACLF is considered to be the most challenging and intractable condition in HBV-cirrhosis patients. For rapid disease progression and high mortality [18, 22], we sought to determine whether C3 levels can predict clinical outcomes of ACLF. Compared with current predictive score models, C3 levels did not seem to be an ideal predictor of HBV-C-ACLF outcomes. Despite this, we still observed elevated plasma C3 levels in conjunction with recovery in HBV-C-ACLF patients. It should be noted that the predictive ability was calculated based on the C3 levels measured at admission. However, ACLF is often unstable and evolutionary, so longitudinal monitoring remains important. The mechanisms by which C3 levels returned to normal in survivors also require further investigation.

Overall, the development of modern precision medicine highlights the importance of more integrated and accurate biomarkers for disease management [28, 29]. The complement system is often neglected in the clinical diagnosis and treatment of liver diseases, but this study revealed that C3 levels are valuable in assessing the severity of CHB-related stages. More importantly, low C3 levels signifies the development of ACLF in patients with decompensated cirrhosis. Based on the data, we believe that sequential measures of serum C3 levels in patients with AD of cirrhosis will provide a more accurate diagnosis of disease progression or deterioration, and provide better guidance for interventions. However, this study still had several limitations. Firstly, CHB patients with severe symptoms and higher transaminases or bilirubin levels were more likely to be admitted and included in our analyzed cohort due to the follow reasons: (1) CHB patients usually receive outpatient care, and patients with mild symptoms or asymptomatic cases do not need hospitalization; (2) Shanghai Public Health Clinical Centre is a regional tertiary hospital for infectious diseases in eastern China, most of its

admitted CHB patients have severe symptoms or higher abnormal indicators than regular medical institutions; and (3) recently, due to the impact of the COVID-19 epidemic, the inpatient beds in the Department of General Liver Disease have been reduced. While, the CHB status might affect the C3 levels as well. The lack of the data from milder cases precluded further analysis of this issue. Secondly, the retrospective analysis hindered the mechanistic explanation or causality of C3 decline in HBV-AD patients. Thirdly, we only analyzed serum levels of C3 and did not further analyze its expression in liver tissue. To this end, we have been planning to study a larger cohort from multi-center medical institutions and looking for opportunities for further deep analysis of the C3 expression in liver tissue in an upcoming study.

CONCLUSION

Through analysis of the C3 data from a wide range of CHB patients at different stages, the concise C3 signatures have been described. We found that serum C3 levels are valuable in assessing the severity of CHB-related stages. Low C3 levels can also alert the development of ACLF in patients with decompensated cirrhosis.

ACKNOWLEDGEMENTS

We are indebted to all the staff members responsible for the clinical database for their assistance in data acquisition.

Funding. Sponsorship for this study, and Rapid Service Fees, were funded by Shanghai Hospital Development Center Foundation (SHDC12020109), Shanghai Association for Science and Technology (21S11905600), Plan of the research Project funded by Shanghai Public Health Clinical Centre (KY-GW-2022-20) and National Natural Science Foundation of China (81974259, 82172250).

Medical Writing/Editorial Assistance. We also thank for the language editing service from MedE Medical Editing Group Inc. Shanghai Hospital Development Center Foundation (SHDC12020109) who provided the assistance and the source of funding for this assistance.

Authorship. All named authors meet the International Committee of Medical Journal Editors (ICMJE) criteria for authorship for this article, take responsibility for the integrity of the work as a whole, and have given their approval for this version to be published.

Author Contributions. Chong Chen, Zhu Yuan, Weixia Li, Ling Fei, Liujuan Ji, Qin Huang, Shuye Zhang and Liang Chen contributed to the material preparation and data analysis and/or interpretation. All authors read and approved the final manuscript and are accountable for accuracy and integrity of the data presented therein.

Disclosures. Chong Chen, Zhu Yuan, Weixia Li, Ling Fei, Liujuan Ji, Qin Huang, Shuye Zhang and Liang Chen all approved the final version of the manuscript and declare no conflicts of interest.

Compliance with Ethics Guidelines. The study was reviewed and approved for publication by the Institutional Review Board of the Shanghai Public Health Clinical Centre (NO.2022-S075-01). The requirement for individual written informed consent was waived since the study was retrospective in design, all patient information was anonymous and only currently existing data was used. We complied with the Helsinki Declaration 1964, and its later amendments.

Data Availability. The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

REFERENCES

1. Nguyen MH, Wong G, Gane E, Kao JH, Dusheiko G. Hepatitis B virus: advances in prevention,

diagnosis, and therapy. Clin Microbiol Rev. 2020. https://doi.org/10.1128/CMR.00046-19.

2. EASL. Clinical practice guidelines on the management of hepatitis B virus infection. J Hepatol. 2017;2017(67):370–98.

3. Terrault NA, Lok A, McMahon BJ, Chang KM, Hwang JP, Jonas MM, et al. Update on prevention, diagnosis, and treatment of chronic hepatitis B: AASLD 2018 hepatitis B guidance. Hepatology. 2018;67:1560–99.

4. Tsochatzis EA, Bosch J, Burroughs AK. Liver cirrhosis. Lancet. 2014;383:1749–61.

5. Arroyo V, Moreau R, Jalan R. Acute-on-chronic liver failure. N Engl J Med. 2020;382:2137–45.

6. Mathern DR, Heeger PS. Molecules great and small: the complement system. Clin J Am Soc Nephrol. 2015;10:1636–50.

7. Ostrycharz E, Hukowska-Szematowicz B. New insights into the role of the complement system in human viral diseases. Biomolecules. 2022. https://doi.org/10.3390/biom12020226.

8. Elvington M, Liszewski MK, Bertram P, Kulkarni HS, Atkinson JP. A C3(H20) recycling pathway is a component of the intracellular complement system. J Clin Invest. 2017;127:970–81.

9. Mastellos DC, Pires DSB, Fonseca B, Fonseca NP, Auxiliadora-Martins M, Mastaglio S, et al. Complement C3 vs. C5 inhibition in severe COVID-19: early clinical findings reveal differential biological efficacy. Clin Immunol. 2020;220: 108598.

10. Chen Z, Diaz G, Pollicino T, Zhao H, Engle RE, Schuck P, et al. Role of humoral immunity against hepatitis B virus core antigen in the pathogenesis of acute liver failure. Proc Natl Acad Sci USA. 2018;115:E11369–78.

11. Klein AD, Gonzalez DLVJ, Zanlungo S. Complement component C3 participates in early stages of niemann-pick C mouse liver damage. Int J Mol Sci. 2020. https://doi.org/10.3390/ijms21062127.

12. Monsinjon T, Gasque P, Chan P, Ischenko A, Brady JJ, Fontaine MC. Regulation by complement C3a and C5a anaphylatoxins of cytokine production in human umbilical vein endothelial cells. Faseb J. 2003;17:1003–14.

13. Zhang GL, Zhang T, Ye YN, Liu J, Zhang XH, Xie C, et al. Complement factor 3 could be an independent risk factor for mortality in patients with HBV related acute-on-chronic liver failure. Biomed Res Int. 2016;2016:3524842.

14. Li Q, Lu Q, Zhu MQ, Huang C, Yu KK, Huang YX, et al. Lower level of complement component C3 and C3a in the plasma means poor outcome in the patients with hepatitis B virus related acute-on-chronic liver failure. Bmc Gastroenterol. 2020;20: 106.

15. Lefton HB, Rosa A, Cohen M. Diagnosis and epidemiology of cirrhosis. Med Clin North Am. 2009;93:787–99.

16. Wu T, Li J, Shao L, Xin J, Jiang L, Zhou Q, et al. Development of diagnostic criteria and a prognostic score for hepatitis B virus-related acute-on-chronic liver failure. Gut. 2018;67:2181–91.

17. Li J, Liang X, You S, Feng T, Zhou X, Zhu B, et al. Development and validation of a new prognostic score for hepatitis B virus-related acute-on-chronic liver failure. J Hepatol. 2021;75:1104–15.

18. Khanam A, Chua JV, Kottilil S. Immunopathology of chronic hepatitis B infection: role of innate and adaptive immune response in disease progression. Int J Mol Sci. 2021. https://doi.org/10.3390/ijms22115497.

19. Sarin SK, Choudhury A. Acute-on-chronic liver failure: terminology, mechanisms and management. Nat Rev Gastroenterol Hepatol. 2016;13: 131–49.

20. Gao B, Jeong WI, Tian Z. Liver: an organ with predominant innate immunity. Hepatology. 2008;47: 729–36.

21. Lung T, Sakem B, Risch L, Wurzner R, Colucci G, Cerny A, Nydegger U. The complement system in liver diseases: evidence-based approach and therapeutic options. J Transl Autoimmun. 2019;2: 100017.

22. Philips CA, Ahamed R, Abduljaleel JK, Rajesh S, Augustine P. Critical updates on chronic hepatitis B virus infection in 2021. Cureus. 2021;13: e19152.

23. Sarin SK, Choudhury A, Sharma MK, Maiwall R, Al MM, Rahman S, et al. Acute-on-chronic liver failure: consensus recommendations of the Asian Pacific association for the study of the liver (APASL): an update. Hepatol Int. 2019;13:353–90.

24. Kamath PS, Kim WR. The model for end-stage liver disease (MELD). Hepatology. 2007;45:797–805.

25. Ruf A, Dirchwolf M, Freeman RB. From Child-Pugh to MELD score and beyond: taking a walk down memory lane. Ann Hepatol. 2022;27: 100535.

26. Engelmann C, Claria J, Szabo G, Bosch J, Bernardi M. Pathophysiology of decompensated cirrhosis: portal hypertension, circulatory dysfunction,

inflammation, metabolism and mitochondrial dysfunction. J Hepatol. 2021;75(Suppl 1):S49–66.

27. Zaccherini G, Weiss E, Moreau R. Acute-on-chronic liver failure: definitions, pathophysiology and principles of treatment. JHEP Rep. 2021;3: 100176.

28. Duraisamy GS, Bhosale D, Lipenska I, Huvarova I, Ruzek D, Windisch MP, et al. Advanced therapeutics, vaccinations, and precision medicine in the treatment and management of chronic hepatitis B viral infections; where are we and where are we going? Viruses. 2020. https://doi.org/10.3390/v12090998.

29. Ginsburg GS, Phillips KA. Precision medicine: from science to value. Health Aff (Millwood). 2018;37:694–701.