ABSTRACTS

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Abstract 001

What Causes Anemia in Admitted Young Adults? A Cross-Sectional Study from a Tertiary Care Hospital in Eastern India

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Introduction Anemia is the most common hematological alteration in medicine wards. Often, the diagnosis remains elusive and blood transfusion for alleviation of symptoms is sometimes resorted to. The present etiological study of anemia was undertaken in a tertiary care hospital to find a cost-effective way to approach a case of anemia in a young adult in our setup. *Materials and methods*: Patients of both sexes, aged between 12 and 40 years, admitted with anemia (with exclusion of other illnesses) in medicine wards were included in the study. They were subjected to a pre-designed study protocol. All tests were done before any blood transfusion or at least 3 months after the last blood transfusion. For analysis the patients were divided into three age groups: <20, 20–30, and 30–40 years. *Result* A total of 106 patients were included in the study, of which 39.62% ($n = 42$) were female. 37.73% ($n = 40$) were smokers and all of them were males. The mean age of the patients was 29.12 ± 8.3 years; the youngest patient being of 13 years and the eldest 40 years. Among the different age groups, non-infective causes predominated in youngest (<20 years) age group (85.71%), while in the 20–30 year age group, 50% ($n = 8$) were infective. Among the male population, 26.47% ($n = 18$) had an infective etiology while in females, 23.8% ($n = 10$) had an infective cause. Of the infective causes, tuberculosis was the commonest cause ($n = 10$, 35.71%), while in the non-infective group, liver diseases was the commonest ($n = 20$, 25.64%). Logistic regression analysis showed globulin levels correlated with increased chance of an infective etiology (OR = 2.1066, CI = 3.5561, $P = 0.0053$). Age-group wise analysis of hemoglobin levels showed the youngest group (<20 years) was the most susceptible (Mean Hb 6.41 ± 2.605 g/dl). *Discussion* The present study shows the diverse etiologies of anemia in young adults. The age group <20 years is the most susceptible to anemia, especially from an infective cause. In our setup, infective etiology is still the main culprit; however, liver diseases, especially post-alcoholic are also an important cause of anemia. Some factors like serum globulin level could be included in early investigation protocols of anemia to give a clue to the etiology.

Abstract 002

Immunohaemolytic Anaemia in Lymphoproliferative Disorders

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The study was done to highlight the incidence of immunohaemolytic anaemia in lymphoproliferative disorders. Five cases of immuno-haemolytic anaemia were diagnosed during the last 6 years. Age ranged from 43 to 69 years, with four males and one female. They presented with lymphadenopathy, splenomegaly and pallor and were placed in Grade III. EDTA blood was collected for complete hae-mogram, blood smear examination, reticulocyte count was done routinely, bilirubin (total, conjugated and unconjugated) was done. DAT, serum LDH and CD5 antigen were done. All five patients showed evidence of haemolytic anaemia by presence of normoblasts, polychromatophilia, reticulocytosis, DAT positive, elevated serum LDH and CD5 positive. Four cases were Chronic Lymphatic Leu-kaemia and one a case of low grade Small Lymphocytic Lymphoma. Approximately 8–25% patients develop immunohaemolytic anaemia as a complication of lymphoproliferative disorders. CD5 positive lymphocytes secrete anti DNA antibodies and rheumatoid factors and play significant role in producing auto antibodies against red cell leading to predominantly warm antibody type of immunohaemolytic anaemia. CD5 positive malignant clone are also responsible for pure red cell aplasia, immunothrombocytopenias, autoimmunoglomerulonephritis (nephrotic syndrome), autoimmune blistering skin disease and paraneoplastic pemphigus.

Keywords Immunohaemolytic anaemia, Lymphoproliferative disorders, CLL, Low grade SLL, DAT, LDH, CD5

Abstract 003

Multiple Myeloma Presenting as Fever of Unknown Origin in a Young Male

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Case Report Plasma cell dyscrasia is usually a disease of old age, this is characterized by neoplastic proliferation of plasma cell with production of monoclonal immunoglobulin. Among these malignant conditions there are multiple myeloma, plasmacytoma, etc. Intermediate is smoldering multiple myeloma and benign is monoclonal gammopathy of undetermined significance. We hereby report a case of a 28-year male presented with fever of unknown origin (FUO) for 6 weeks. On examination he had pallor and mild jaundice; there was no hepato splenomegaly and lymphadenopathy. Malaria double antigen, Widal test, Ig-M for *S. typhi* and HIV-I and II all of these were negative. In routine blood test patient was found to have normocytic normochromic anemia with high ESR (165 mm/h). Differential count showed lymphocytes 15%, plasma cells 8%. Aldehyde test was strongly positive. In bone marrow examination it was hypercellular with decreased erythropoiesis and plasma cell was >40%, plasma cells were atypical binucleate, few were flame cells and plasma blast. No LD body was found. From this findings possibility of plasma cell dyscrasia was considered. In biochemical tests—urea, creatinine, SGPT were increased. Unconjugated bilirubin was also increased. Globulin was high and A:G was 0.7:1. Alkaline phosphatase was mildly increased. Urine Bence Jones protein was positive. Patient was sent for serum electrophoresis and M spike was found, it was 4.06 gm/dl in gamma region. In X-ray skull only one lytic lesion was found. After this patient was sent for bone scan there was increased uptake in ribs and other bones. Patient was diagnosed as multiple myeloma and treated in Radiotherapy Department with melphalan and steroids and he was responding well.
Keywords Multiple myeloma, Young male, FUO

Abstract 004

Severe Anaphylactic Reaction in IgA Deficient Patient Following Transfusion of Whole Blood

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Background IgA anaphylactic reaction is a rare event, estimated to occur 1 in 20,000–47,000 transfusions. Although the reaction is rare, the clinical manifestation sometimes is so severe and even life threatening. We report a case of severe anaphylactic reaction in a patient with IgA deficiency following transfusion of whole blood. Case discussion: A 56-year-old female admitted in the hospital with complaint of upper G.I. bleed. Her haemoglobin dropped to 6 gm/dl. Her physician advised two units of red cell transfusion on emergency. So, two units of whole blood of same group were cross matched and one unit was issued from the blood bank for immediate transfusion. However, transfusion of as little as 10 ml of blood, she immediately developed light headedness, chest pain, nausea followed by flushing of face and upper part of chest, severe hypotension and laryngeal edema. The symptom lasted for few minutes. The patient was treated conservatively with hydrocortisone and antihistamines. On the following day, she again developed very severe form of anaphylactic reaction immediately after transfusion of 10–15 ml of second unit of whole blood. The clinician referred this patient to our department for evaluation of transfusion reaction and immediate possible solution for safe blood transfusion. In our center, two units of packed red cells of same blood group were cross matched. Before issuing the first unit, the bag was washed three times with normal saline and transfused slowly under medical supervision. Her transfusion was uneventful. The second unit of packed red cell was again washed and transfused without any adverse episode. The patient had active duodenal ulcer, she subsequently required four units of packed red cell units. Each

time she received washed red cell for transfusion and her transfusion was completely successful without any adverse event. The features of transfusion reaction were very much suggestive of severe anaphylactic reaction possibly due to anti-IgA antibody in IgA deficient individual. Her blood sample was tested for estimation serum IgA and anti-IgA antibody. Anti-IgA estimation could not be done as this is a specialized test and not performed routinely. However, she had almost undetectable amount of IgA (<0.05 mg/dl). Discussion: The best documented anaphylactic reactions result from presence of anti-IgA antibodies in IgA deficient patients who receive of blood or blood component containing donor plasma. More evidence based research work is required in patients with anaphylactic reaction to blood or blood components with a view to determine the role of IgA deficiency. Preparing a rare donor panel of IgA deficient blood donors would be good alternative option for patients who experience severe anaphylactic reactions from blood or blood components.
Keywords Anaphylactic, IgA

Abstract 005

Lupus Anticoagulant and Anticardiolipin Antibodies in Systemic Lupus Erythematosus: Prevalence and Clinical Associations

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Background Lupus anticoagulant and anticardiolipin antibodies in SLE have been seen to be associated with thromboembolism, thrombocytopenia and fetal loss in patients with Systemic Lupus Erythematosus (SLE). 84 patients of SLE were tested for presence of Lupus anticoagulant using three assays, APTT with a LA-sensitive reagent, Kaolin clotting time (KCT) and Dilute Russel Viper Venom time (dRVVT). Anticardiolipin antibodies were studied by ELISA. Results: Lupus anticoagulant was present in 17 (20.2%) patients. The LA positive group showed higher number of patients having renal involvement, lymphadenopathy and statistically significant association in patients with trombotic complications and fetal loss but not with thrombocytopenia. Anticardiolipin antibodies were tested in 58 of these patients and were positive in 32.7%. Clinical characteristics were not significantly different between ACL negative and ACL positive groups. However, the patients having thrombotic complications and foetal loss showed statistically significant association with IgM ACL, but not with IgG ACL. Conclusion: Lupus anticoagulant and anticardiolipin antibodies in Systemic Lupus Erythematosus showed different prevalence. Lupus anticoagulant and IgM ACL showed significant associations with thrombotic complications.

Abstract 006

Prevalence of Iron Deficiency in Beta Thalassemia Minor

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Background Iron deficiency anemia is the most common hematological condition worldwide as per WHO survey. Beta thalassemia minor is a condition in which one allele carrying mutation for thalassemia. The state of West Bengal has the highest prevalence for beta thalassemia mutations in India. Hence we planned to study prevalence of iron deficiency in these patients. *Aim of the Study* To assess the iron

status in thalassemia minor subjects. *Methodology* Study design: Prospective, non-interventional, observational study. *Parameters* Prospective collection of demographic, clinical, diagnostic, and laboratory data. Setting: Outpatient, in patient and day care services of Department of Hematology, N.R.S. Medical College and hospital, Kolkata. Criteria for diagnosis of thalassemia minor Hb A2 ≥4.0–10.0% along with low MCV (<80 fl), low MCH (<27 pg). Exclusion criteria: (1) patients suffering from fever; (2) patients with clinically suspected inflammatory disorders and (3) other haemoglobinopathies. *Methodology* All patients underwent meticulous clinical evaluation followed by complete hemogram, HPLC and serum ferritin assays. *Results* Total number of subjects was 150 of which 59 were men (39.33%). Mean age was 33.59 ± 0.67 (6–57). 96.66% were in lower socioeconomic group. The commonest presenting feature was pallor (48%) followed by generalized weakness (29.33%). The mean hemoglobin was 10.94 ± 0.21, MCV was 66.61 ± 1.33, MCH was 20.49 ± 0.40 and RDW (%) was 16.34 ± 0.32.29 (33.05%) had serum ferritin levels less than 15 ng/l, which was considered as cut off for iron deficiency, of these 27 were women. The mean hemoglobin was 9.78 ± 0.19, 11.21 ± 0.22 ($P < 0.01$), MCV 66.24 ± 1.32, 66.70 ± 1.33 ($P > 0.05$), MCH was 20.18 ± 0.4, 20.56 ± 0.41 ($P < 0.01$), RDW was 16.34 ± 0.32, 16.4 ± 0.32 ($P > 0.05$) among subjects with and without iron deficiency state respectively. Hb F levels among beta thalassemia minor was ≤1 in 124(82.66%), 1–2 in 18(12%) and 2–6 in 8(5.3%) subjects. *Conclusions* (a) One of the commonest cause for anemia in beta thalassemia minor is iron deficiency which is often overlooked, (b) women carriers suffer from iron deficiency more than men and (c) 17.3% subjects had >1 HB F.

Abstract 007

Basal Ganglia Infarct in Sickle Cell Disease

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Introduction CVA in a frequent complication in elderly. In children it is uncommon. It is a rare presentation in sickle cell disease (SCD) responsible for various neurologic deficits and mortality. *Case History* A 10-month-old male child presented with left side hemiparesis with facial palsy after a fall. There was no h/o seizure, vomiting, altered sensorium or drug intake. CT Scan of brain revealed ischemic infarct involving basal ganglia. Investigations like routine haemogram, bleeding and coagulation profile were normal except positive sickling test and homozygous (SS) for SCD in HPLC. *Discussion* SCD is a rare cause of a CVA in infants and young children. Ischemic attacks are secondary to occlusion of cerebral vessels and distal field insufficiency, implicated in pathogenesis. *Conclusion* In infants and young children with neurological deficits screening test for SCD is warranted.
Keywords Sickle cell disease, Basal ganglia infarct, HPLC

Abstract 008

A Case of Hairy Cell Leukemia: Variant (HCL-V) Complicated with Opportunistic Infection

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Introduction A variant form of hairy cell leukemia was first described by Cauley and colleagues in 1980 and subsequently termed the prolymphocytic variant of HCL. Representing 0.4% of chronic lymphoproliferative disorder and an estimated 10% of HCL. It resembles HCL but exhibits distinct cytohaematologic and immunophenotypic features, often lack of staining for TRAP and negative for CD25. *Case History* A 45 years HM presented with pancytopenia and huge splenomegaly and was diagnosed as HCL-variant by flow cytometry 0.15 days after receiving chemotherapy he developed epistaxis and hemoptysis. X-ray and CT scan showed large soft tissue lesion with cavity formation and b/l pleural effusion. Subsequently sputum AFB was positive and bronchial lavage showed hypae of aspergillosis. HCL-v usually carries a bad prognosis but this patient is doing well with maintenance therapy and has had no recurrence of symptoms. *Conclusion* Opportunistic infections following chemotherapy can make the situation more complicated awareness of the condition is important in order to make a correct diagnosis.
Keywords HCL-v, Aspergillosis

Abstract 009

Study of Serum Hepcidin Levels in Polytransfused β-Thalassemia Major Patients

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Background Hepcidin, a key regulator of iron homeostasis, is increased by iron overload and inflammation while anemia and hypoxia induce its suppression. In spite of iron overload in β-thalassemia major (β-TM), a paradoxical decrease in hepcidin is observed. *Aim of the Study* To assess the opposing effects of enhanced erythropoiesis due to anemia and iron overloading on hepcidin as their common target, hepcidin levels in β-TM were analyzed and correlated with serum transferrin receptors (sTfR) and serum ferritin. *Methodology* Eighty three paediatric patients of β-TM (Group A) on regular transfusion and chelation, along with 70 controls (Group B) were taken, excluding those with <20 blood transfusions, deranged liver and renal function test, evidence of Hepatitis B, C and HIV infection and C-Reactive Protein >8 mg/l. Complete blood count was analysed by KX-21, serum assay for ferritin, transferrin receptors and hepcidin was done by ELISA. Data was analysed by relevant statistical analysis. *Results* The age ranged from 1.5 to 18 years with male: female ratio 0.97:1 for Group A (0.75:1 in Group B). Serum ferritin, a marker for assessing iron overload, was found to be significantly elevated in Group A as compared to Group B (3428.80 ± 1741.65 ng/ml vs. 107.0 ± 121.31 ng/ml; *P* value <0.001). Similarly, mean sTfR, reflecting the degree of erythropoiesis, was significantly higher in Group A than Group B (5.18 ± 2.58 µg/ml vs. 3.60 ± 2.26 µg/ml respectively; *P* value <0.001). However, serum hepcidin levels were found to be comparable (13.88 ± 10.68 ng/ml vs. 14.47 ± 11.68 ng/ml respectively; *P* value = 0.786). A significant negative correlation was found between serum hepcidin and sTfR (*P* value <0.0066). However, there was no correlation of serum hepcidin with serum ferritin ($r = -0.009$, $P = 0.408$). The ratio of serum hepcidin to ferritin, an indicator of appropriateness of response to elevated serum ferritin, was found to be in a range of 0.000127–0.0272 (mean 0.00552) in β-TM patients while it was 0.0023–1.893 (mean 0.378) in group B. The difference was statistically significant (<0.001). *Conclusion* In conclusion, the increased iron demand has an upper hand over iron overload in regulating the levels of hepcidin in β-TM patients. In spite of excessive iron load, the inappropriate levels of hepcidin may further contribute to iron overload and thus enhance iron toxicity. So, it can be used as a potential therapeutic target.

Abstract 010

A Spectrum of PNH: A Study of 5 Cases

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Introduction Paroxysmal nocturnal haemoglobinuria (PNH) is an acquired stem cell disorder with deficiency of GPI anchor resulting in accelerated complement mediated hemolysis of red cells. Classically has a triad of clinicohematological presentation of cytopenias, hemolysis and thrombosis with paroxysmal haemoglobinuria in only 25%. Also a strong association with aplastic anemia (30%) remains a remarkable feature. *Methods* Five cases of PNH with varied clinical presentations and progression have been studied here. Two cases presented with pancytopenia and hemolysis. One case had a preceding h/o aplastic anemia with subsequent development of PNH. One case was a young man with unexplained anemia which was diagnosed later as PNH. Last case was a 8-year-old child with refractory anemia, later diagnosed as PNH and followed up for 6 years but succumbed to repeated infections. All the cases were diagnosed by flow cytometry showing variable reduction in CD55 and CD59. *Conclusion* Progression into PNH from aplastic anemia must be kept in mind when evaluating and following up a case of aplastic anemia. Flowcytometric analysis remains the gold standard not only for diagnosis but for follow up of PNH.
Keywords PNH, Flow cytometry, Aplastic anemia

Abstract 011

PLZF RARα Variant of Acute Promyelocytic Leukemia (APL): A Case Report and Review of Literature

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A 23-year-old male presented with history of fever and shortness of breath since last 1 month. Physical examination revealed an area of dullness on chest examination and mild hepatosplenomegaly. The chest X-ray revealed bilateral pleural effusion. The pleural tap was hemorrhagic and showed myelocytes and metamyelocytes. Hemogram showed mild anemia with a hemoglobin of 9.2 g/dl, a high TLC of 19,100/μl and adequate platelet count. The differential count was P36, L24, M01, E01, PM10, My22, MM06. Many Pseudo Pelger huet cells were seen. The coagulation profile was deranged with a positive D-dimer test. Doppler ultrasound of lower limbs was done to rule out thrombosis and was non contributory. The bone marrow examination was done in view of anemia and immature cells in peripheral blood. BM aspirate and biopsy revealed hypercellular marrow spaces. Myeloid to erythroid ratio was increased with 52% cells being promyelocytes. These promyelocytes had regular round to oval nuclei, moderate amount of cytoplasm with few granules and occasional Auer rods. These cells were smaller than usual promyelocytes. However cytochemical stains performed on the BM aspirate smear showed intense staining of most of these cells with MPO and Sudan black B (SBB) confirming to the morphology of promyelocytes. As there was massive maturation arrest, other causes related to infections (Pseudomonas) and drug intake were ruled out. Cytogenetics analysis of the BM aspirate revealed an apparently balanced t(11;17)(q23;q21) in all 20 of the examined G-banded cells. Final diagnosis rendered was APL (PLZF RARα). The patient was lost to follow up after diagnosis for 3 months and returned after taking some desi medicines. The follow up hemogram showed similar findings. He refused chemotherapy and succumbed to his illness after 1 month. Acute promyelocytic leukemia (APL) is a distinct subtype of acute myeloid leukemia. Cytogenetically, APL is characterized by a balanced reciprocal translocation between chromosomes 15 and 17, which results in the fusion between the promyelocytic leukemia (PML) gene and retinoic acid receptor (RAR). Variant chromosomal translocations [e.g., t(11;17), t(5;17)] can be detected in no more than 2% of APL patient. This case illustrates the importance of correlating unusual features of promyelocytes i.e. rounded eccentric nuclei, few granules and occasional Auer rod along with pseudo pelger-huet cells in conjunction with cytogenetic findings, while evaluating a case of acute promyelocytic leukemia. Differentiation of zinc finger variant from other variants of APL is critical as it is resistant to arsenic trioxide and ATRA as a single-agent therapy.

Abstract 012

The t(8;21)(q22;q22) in Myeloid Malignancies in India

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Background The t(8;21)(q22;q22) is the most common translocation seen in acute myeloid leukemia (AML). It occurs in 10–15% of all AML and is associated with a favourable outcome. The AML1/ETO fusion gene which results from the translocation blocks the normal differentiation of granulocytes. This study describes the cytogenetic features of t(8;21)AML. There is limited data on this subset of AML from India. *Patients and Methods* All patients with the t(8;21) seen in the Department of Haematology, Christian Medical College, Vellore between January 2003 and May 2010 were studied. G-banded karyotypes were reported according to the International System for Human Cytogenetic Nomenclature (ISCN) 2005. Karyotype findings were correlated with bone marrow morphology as well as immunophenotype and molecular genetic analyses when available. *Results* The t(8;21) was seen in 130 patients, Association of Mean Platelet Volume and Ischemic Stroke. 129 of whom had AML (9% of 1424 AML). The remaining patient who was on Imatinib for Philadelphia chromosome positive chronic myeloid leukemia developed the t(8;21) in blast crisis. The median age was 23 years (range 2–72). There were 88 adults (68%) and 82 males (63%) in this series. The bone marrow showed M2 morphology in 105 patients (80%), AML-NOS (not otherwise specified) in 14 (12.2%), M1 in seven (5.5%), M4 in two (1.5%) and M5 in one (0.76%).The marrow blast count was 2–88%. Eight (7.6%) AML-NOS had less than 20% blasts. The WBC index was available in 87 patients. The median WBC index was 4.2 (range 0.13–159). Dysplasia was noted in 84 patients (65%) and eosinophilia in 24 (18%). Immunophenotype (72 patients) showed expression of HLA-DR in 96%, CD34 in 86% and CD19 in 54% and CD56 in 6 of 10 patients. Mutation analysis of 29 patients showed heterozygosity for FLT3-ITD, FLT3-TKD and CKIT D816V mutations in one patient each (3%). None of the patients showed NPM1 mutation. A solitary t(8;21) was seen in 20 patients (15%). Eighty (60%) had a single additional abnormality and 30 (25%) had two or more additional abnormalities (complex karyotypes).The most frequent additional abnormality was loss of a sex chromosome in 85 patients (65%) followed by deletion (del) of the long (q) arm of chromosome 9 in 23 (17%); 11 patients (8%) had both these abnormalities. Other abnormalities were less frequent and were seen in 34 (24%) patients. These included trisomies 4, 8 and 15, monosomy 17, del 5q and del or addition 7q, each occurring in

less than 5% of patients. Non-recurrent clonal abnormalities were seen in 10% of patients. Three or four-way variant t(8;21) were seen in eight patients (6%). Five (4.6%) had tetraploid karyotypes. *Conclusion* Our findings are similar to previous reports from other countries with respect to the overall incidence (9%) of the t(8;21) and its association with additional abnormalities (85%), loss of a sex chromosome (65%), AML-M2 (80%) and expression of CD19 (54%) and CD56 (60%). The incidence of FLT3-ITD, FLT3-TKD and CKIT D816V mutations (3% each) is lower than what has been reported. This is the first detailed analysis of t(8;21) AML from India. Correlation of the variations within this cohort with treatment outcome would be important to understand.

Abstract 013

Chronic Neutrophilic Leukaemia: A Case Report

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Background Chronic neutrophilic leukaemia (CNL) is a rare chronic myeloproliferative disorder of elderly presenting as sustained mature neutrophilic leucocytosis with few or no circulating immature granulocytes. It has been accepted recently as a distinct entity by World Health Organisation classification of hematopoietic malignancies. Till date 129 cases are reported in the literature. *Aims* To report the finding from a case of chronic neutrophilic leukaemia. *Methods* We report a case of 63-year-old male presenting with complaints of fatigue and abdominal discomfort for past 3 months. Clinical examination showed enlarged liver 2 cm below right subcostal margin and spleen 5 cm below left subcostal margin. Investigation including routine blood examination and bone marrow examination was carried out. Later NAP score and PCR for bcr-abl was done. *Results* Blood examination showed haemoglobin 15 gm%, White blood cell count 34.2×10^9/l, differential count—neutrophil 87%, lymphocyte 6%, basophil 3%, eosinophil 4%. Platelet count—260×10^9/l. Absolute neutrophil count was 29.7×10^9/l. Bone marrow examination showed hypercellular marrow with marked neutrophilic proliferation. Neutrophil Alkaline Phosphatase was elevated. PCR study for bcr-abl came out negative. Conclusion: So the diagnosis of CNL was offered. **Keywords** Chronic neutrophilic leukaemia, Myeloproliferative disease, PCR

Abstract 014

Pancytopenia in Chronic Lymphoproliferative Disorders

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Chronic lymphoproliferative diseases (CLPD) include a heterogenous group of disorders with a unique biology and clinical course. These disorders can pose a diagnostic challenge when they present with either unusual clinical or laboratory features. *Aim* To present three cases of CLPD that presented as pancytopenia. Case 1—50 year female presented with severe pallor, fatiguability and giddiness since 5–6 months. She had received blood transfusions 4–5 times for her complaints. She had a past history of splenectomy, hysterectomy and appendectomy. Splenectomy was done 5 years back, details of the operation were not known. Her hemogram was Hb 5 g/dl, TLC 3,800/mm³ and Platelets 7,000/mm³. DLC was P-08, L-92. Bone marrow was diluted with increased lymphocytes. Few lymphoplasmacytoid cells were also seen. Coombs test was negative. On immunophenotyping of bone marrow

aspirate a diagnosis of chronic lymphocytic leukemia was made. Case 2—47 year male presented with fever and bodyache of 6 months duration. He had received 4 blood transfusions. On examination there was no organomegaly. His hemogram was Hb 8.2 g/dl, TLC 3,000/mm³ and Platelets 63,000/mm³. DLC revealed P2 L98. A provisional diagnosis of Aplastic anemia was made. Bone marrow aspirate was diluted. A bone marrow biopsy revealed small lymphoid cells in sheets. On immunohistochemistry the case was LCA, CD5, CD20 and Cyclin D 1 positive and CD23 negative. A diagnosis of mantle cell lymphoma was made. Case 3—50 year old male presented with complaints of fatigue and weakness of 1 year duration. He had also difficulty in breathing. On examination he had generalised lymphadenopathy and pleural effusion. His Hb was 8.3 g/dl, TLC 1,400/mm³ and Platelet 1 lakh/mm³. Peripheral smear showed a few lymphocytes. Pleural fluid cytology revealed only lymphocytes. A provisional diagnosis of MDS with pleural effusion was made. Bone marrow aspirate revealed mainly megaloblastic erythroid hyperplasia with few atypical lymphoid cells. Bone marrow biopsy showed atypical lymphoid cells with fried egg appearance. On immunophenotyping these few atypical lymphoid cells were strong CD20, CD25 and CD103 positive. A diagnosis of Hairy cell leukemia was made. *Conclusion* Chronic lymphoproliferative disorder presenting as pancytopenia can be difficult to diagnose. It needs an expert morphological assessment along with advanced ancillary techniques to render a correct diagnosis.

Abstract 015

Alpha Thalassemia Deletion Mutations and Their Coexistence with Hemoglobinopathies in the Santhals of West Bengal

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Background The hemoglobinopathies are autosomal recessive conditions affecting the quantity and quality of hemoglobin molecules which are found in certain ethnic groups. In West Bengal, Santhals represent 54.27% of total tribal population. The alpha thalassemia carrier rates in India vary from 1 to 80%. Coexistence of alpha thalassemia deletion in beta thalassemia patients modifies the phenotypic change. The patients with microcytic, hypochromic anemia and normal HbA2 levels might be misdiagnosed as silent beta thalassemia. α-Thalassemia reaches high frequency (0.35–0.92) in Indian tribal population of Andra Pradesh; in other tribes the frequency is lower (0.03–0.12). Most common molecular basis for alpha thalassemia in India is the geographically widespread 3.7 and 4.2 kb deletions. Their high incidence is reported in tribes of Central, South and West India. α+ is clinically silent in heterozygous and homozygous states, so is free from morbidity. *Aim* Few reports are available on the distribution of different types of hemoglobinopathies and their interaction especially in tribal populations. Our aim was to correlate alpha and beta globin gene mutations in the Santhals. *Materials and Methods* Choice of subjects: The cases belonged to Santhals of West Bengal. They were interrogated about the family history, blood transfusion etc. We analyzed 150 unrelated Santhals. 100 healthy urban people were chosen as the control group. Collection of blood: 5 ml of blood was collected in an EDTA vacutainer and subjected to complete hemogram using an automated cell counter (Sysmex K1000, Japan). Tracing of Beta and E traits: The blood was subjected to hemoglobin electrophoresis at pH 8.6, fetal Hb estimation by alkali denaturation and measurement of HbA2 level respectively. Detection of β-globin mutations: Mutation detection was performed by polymerase chain reaction based amplification refractory mutation system

(ARMS) using genomic DNA for identification of β thalassemia mutations commonly found in Eastern India. Detection of α-globin mutations: For α thalassemia common deletions such as $-\alpha 3.7$ deletion and $-\alpha 4.2$ deletion were identified by single tube multiplex PCR method. *Results* A moderate incidence (10%) of abnormal hemoglobin was detected in 150 Santhals cases. This included 2% beta thalassemia trait and 7.34% HbE trait. One case of ES and two cases of homozygous HbE were detected. Further investigations showed the presence of IVS1–5 (G–C) for beta carriers, Codon26(G–A) for HbE carriers in heterozygous states. High prevalence of both $-\alpha 3.7$ and $-\alpha 4.2$ deletions of alpha globin gene were found as six out of fifteen carriers showed these deletions in heterozygous (2), homozygous (3) and compound heterozygous (1) states. Homozygous $-\alpha 3.7$ frequencies were found to be higher than $-\alpha 4.2$ deletions. The high prevalence of alpha thalassemia homozygotes (7.34%) and compound heterozygotes (18%) indicates multiple recombination events in presence of natural selection of malaria or other environmental factors. The cases with no deletional mutations should be investigated further for the presence of other mutations. *Conclusions* Presence of globin gene defects is common in Indian communities. This study shows the presence of α and β-thal mutations and more prevalence of alpha thalassemia mutations in Santhals of West Bengal.

Abstract 016

Prevalence of Delta–Beta Thalassemia in the Population of Eastern India

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Background Delta–Beta ($\delta\beta$) thalassemia are heterozygous disorders characterized by elevated level of fetal haemoglobin (HbF) and absent or reduced synthesis of adult hemoglobin (HbA) in adult life. Mutations affecting the δ globin gene cause either a reduction ($\delta+$ thalassaemia) or absence ($\delta 0$ thalassaemia) of δ globin synthesis. Although these conditions are clinically silent, when co-inherited with β thalassemia, they prevent an increase in the level of HbA2 which may be confused with the diagnosis of the β thalassemia carrier state. $\delta\beta$ thalassaemia gives rise to Thalassaemia intermedia, the condition which is characterized by a transfusion-independent clinical course of intermediate severity between thalassemia major and asymptomatic carriers. A complex double-deletion/inversion rearrangement has been reported in several Indian families with $(A\gamma\delta\beta)0$ thalassaemias. *Aim and Objective* The aim of the study was to trace out the presence of Asian Indian inversion–deletion $G\gamma$ $(A\gamma\delta\beta)°$ mutation in the cases with elevated level of fetal haemoglobin (HbF) in the population of Eastern India. Materials and Methods: Cases referred to the Haematology unit (I.H.T.M., Kolkata) for investigation of $\delta\beta$ thalassemia were analysed. In our study we had analysed 16 individuals with increased Hb F level out of 350 referred cases. 5 milliliters of blood was collected and haematocrit values were estimated by automated cell counter. HbA2 and HbF levels were measured using cation exchange high performance liquid chromatography. DNA was isolated and Asian Indian inversion–deletion $G\gamma(A\gamma\delta\beta)°$ mutation was investigated by Craig et al. (1994) for the cases with high HbF by PCR technique. *Results* A total 16 (4.57%) individuals with increased HbF level were studied for $G\gamma(A\gamma\delta\beta)°$ mutation out of 350 referred cases. There range of age was between 3 and 38 years, among them 9 were male and 7 were females. Three cases had coexisting beta thalassemia and delta gene mutations resulting in transfusion dependent anemia. Presence of $G\gamma(A\gamma\delta\beta)°$ mutations in heterozygous state was found in 8 (50%) cases and they were absent in 7(43.75%) cases. We did not find any cases with homozygous state of this mutation and one case was uncharacterized. Family studies were carried out on six families (12 individuals) and the mutation was determined for probable future prenatal study. We found six family individuals were heterozygous state for $G\gamma(A\gamma\delta\beta)°$ mutation. Conclusion: The prevalence rate of $\delta\beta$ thalassemia mutation in the studied population was 2.29%. Co-inheritance of $\delta\beta$-thal with β-thal can lead to variable clinical phenotypes. Genotyping of high Hb F determinants are

not frequent in India when they are associated with β-thal or other hemoglobinopathies. Such molecular characterization will help us to provide appropriate counseling and prenatal diagnosis.

Abstract 017

Association of Mean Platelet Volume and Ischemic Stroke

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Introduction and Aim of Study Large platelets are believed to be more reactive, produce more thrombotic factors, and aggregate more easily. Although studies have demonstrated an association between myocardial infarction and the presence of large platelets, very few studies have looked at the association between large platelets and ischemic stroke. Hence we measured mean platelet volume (MPV) in patients with ischemic cerebrovascular disease and age and sex matched controls and evaluated the association between MPV and stroke. *Methods* Between Nov 1st 2008 and July 31st 2009, we prospectively recruited patients who presented to St. John's Medical College Hospital, Bangalore with ischemic stroke. Age and sex matched patients with medical illness (diabetes and hypertension) were recruited as controls. All patients and controls presenting to the medical and neurological wards within 48 h of onset of symptoms and satisfying the inclusion and exclusion criteria were recruited to the study after obtaining informed consent. Clinical severity of stroke was assessed using modified Rankin's scale. A single sample of blood was drawn using a 21 G needle into a vacutainer containing sodium citrate (Becton Dickenson, San Jose, CA). Platelet counts and MPV was measured using an automated hematology analyzer (ABX Pentra, Bioumerieux, France) within 4 h of blood collection. A peripheral blood smear was performed on every sample to ensure that there were no platelet aggregates. *Results* Mean platelet volume was higher in ischemic stroke patients ($n = 50$) when compared to age, sex, and disease matched controls (MPV $= 7.4 \pm 0.8$ fl vs. 6.9 ± 0.6 fl; $P < 0.01$). The platelet count was lower in stroke patients when compared with controls (2.56 ± 0.58 lakh/μl vs. 2.69 ± 0.83 lakh/μl), however this difference was not significant. On multivariate regression analysis, controlling for other variables, MPV was independently associated with stroke (Adj. OR $= 8.1$; $P < 0.009$). We did not find a significant relationship between clinical severity of stroke or sub types of stroke and MPV although this could have been due to a relatively small sample size. *Conclusions* Elevated mean platelet volume is associated with ischemic cerebrovascular disease in this patient population. This observation suggests that large platelets could have a role in the genesis of cerebral thrombosis. Additional research is required to delineate the etiological role of platelet volume in stroke pathology and outcome. More importantly, prospective longitudinal studies may identify those who are at risk for developing stroke.

Keywords Ischemic stroke, Stroke outcome, Platelets, Mean platelet volume

Abstract 018

Reversibility of Renal Failure in Monoclonal Plasma Cell Disorders: The Impact of Novel Agents

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Background Monoclonal plasma cell disorders are common with monoclonal gammopathy of undetermined significance (MGUS) affecting up to 3.2% of all patients over the age of 50 and with multiple myeloma (MM) accounting for 10% of all hematologic malignancies. Renal insufficiency, defined by abnormal creatinine clearance, is present in up to half of myeloma patients at presentation, contributes to excessive early mortality, and diminishes eligibility and clinical outcomes after both systemic therapy and high-dose stem cell transplantation (SCT), as well as novel treatments. Indeed, reversibility of myeloma-associated renal impairment is a critically important prognostic factor and even supersedes response to systemic therapy as a predictor of improved survival. *Aims of the Study* (1) To categorize the cause of renal failure in our study population into Ig dependent or independent mechanism. (2) To find out the impact of novel agents in the reversibility of renal failure due to either of the causes that is Ig dependent or independent. *Methodology* The impact of high dose dexamethasone containing regimens with or without the novel agents Thalidomide, Lenalidomide or Bortezomib on the reversal of renal failure (RF) was evaluated in 26 of 58 consecutive newly diagnosed patients with multiple myeloma (MM) treated in St John's Medical College. Renal failure was defined as a serum creatinine >1.3 mg/dl at the time of diagnosis. Besides antimyeloma treatment, all patients received intensive supportive care including intravenous hydration, alkalinization of urine, correction of hypercalcemia and discontinuation of all potential nephrotoxic agents. Renal dialysis was offered to all patients with an appropriate indication. Reversibility of renal failure which was defined as a sustained decrease of serum creatinine to <1.3 mg/dl. *Results* There were 29 males and 19 females. The mean age of males was 61 and females 54. RF was seen at presentation in 23 (55%). There were 10 Female 15 male patients with renal failure of which RF reversed in 14(73%) within a median of 1.6 months. The mean creatinine at presentation was 5.2 mg/dl and was 1.6 at recovery ($P = 0.035$). Hyperuricemia was associated with a lower probability of RF reversal. Those with RF tended to be 7 years older than those without RF (61 vs. 53 $P = 0.04$) and had a higher BM Plasma cells % 55 vs. 30 ($P = 0.05$). Further analysis shall be submitted at the conference. *Conclusion* RF is reversible in the majority of newly diagnosed MM patients treated with high-dose dexamethasone containing regimens. The addition of novel agents induces a more rapid RF reversal. Initial analysis shows that patients with Ig dependent mechanisms of renal failure showing better response with novel agents.

Abstract 019

Haematological Diagnosis of an Unusual Case of? Clostridial Sepsis

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Background Clostridial sepsis is an almost fatal condition where early diagnosis can be life saving. We report our experience of an unusual case of? Clostridial sepsis without any underlying disease, where timely identification of the haematological features of Clostridial sepsis and prompt institution of therapy helped in saving the patient's life. In India, very few centres have the facility for anaerobic culture, hence all haematologists must recognize these features. *Aim* The aim of this presentation is to spread awareness about the haematological manifestations of Clostridial sepsis. The haematological features of Clostridial sepsis should be recognized by all practicing haematologists who would then alert the clinician of its possibility. *Methodology* A 62-year-old male presented with fever of 1 week duration and unconsciousness since 3–4 h. He was febrile, icteric and unconscious. No source of infection or infecting agent could be identified and the patient deteriorated despite a strong antibacterial and antifungal cover. Renal failure set in. Since no leading history or localizing signs were found, thorough clinical examination was done followed by haematological, biochemical, microbiological and radiological investigations to find the cause. *Results* The haematologist noticed a continuous fall in Hb with features of intravascular haemolysis along with numerous spherocytes on the smear. Spherocytes with intravascular haemolysis in a patient of sepsis suggested Clostridial sepsis. The clinicians were alerted and clindamycin was started promptly. The patient responded within 24 h and became conscious, afebrile and was discharged from the hospital in due course. *Conclusion* Clostridial sepsis is usually identified in the laboratory using microbiological techniques. However, an aware pathologist can also provide a clue to the diagnosis of Clostridial sepsis through a thorough haematological examination, as in this case and help in saving lives.

Abstract 020

"Prothrombin Mumbai" Causes Severe Prothrombin Deficiency due to Novel Cys90Ser Mutation

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Background Homozygous prothrombin deficiency is rare autosomal recessive disorder. Approximately 30 cases of inheritable prothrombin deficiency causing bleeding are reported. The Human Genome Mutation Database features 32 mutations causing prothrombin deficiency. *Aim and Objective* This data is important for the genetic diagnosis of deficiency in affected families. Mutation data of Caucasian, Middle Eastern, Oriental, four patients from South India is reported. It is important to identify new cases particularly so because of the few reports available. We report a rare case of severe congenital prothrombin deficiency leading to severe bleeding in a 4½-month-old female patient from Nepal, with intracranial bleed. *Methods and Results* Baby had several episodes of bleeding. Inj. Vitamin K, Fresh Frozen Plasma and whole blood were given. For laboratory investigations, blood samples of the patient and parents were collected in 3.2% trisodium citrate. Screening coagulation tests of the patient showed APTT 72.2 s, control 27.7 s, mix (1 control:1 patient) 31.4 s. PT was 51.6 s, control 12.4 s, mix 14.1 s. TT was 13.3 s, control of 11.7 s. Plasma Fibrinogen 222 mg/dl. D-Dimer was negative. Factor XIII clot stability test showed stable clot after 24 h. The platelet count was 250×10^9/l, Hb 12.5 g/dl. The coagulation factors were: Factor VC: 76%; Factor VIIC: 68%; Factor VIIIC: 100%; Factor IXC: 54%; Factor XC: 68%; Factor XIC: 88%; Factor XIIC: 66% and Factor IIC: <1% of normal calibrator plasma. Normal range is 50–150%. PCR amplification of all 14 exons in the patient and her parents, followed by DNA sequencing

showed homozygous c.G269C variation in exon 4 of the patient. Both parents were heterozygous for this variation. This variation led to missense Cys90Ser amino acid substitution in the primary protein. Bioinformatics analysis tools ClustalW multiple sequence alignment showed Cys90 is conserved across vertebrate species studied. Ensembl Genome Browser did not show this variation as reported. Polyphen, SIFT and Panther analysis tools predicted this variation as damaging for functionality of the protein. This case adds valuable knowledge to the existing database as we report novel mutation in exon 4 of the pro-thrombin gene. This mutation has caused severe bleeding manifestations in the patient since birth. Subsequent molecular and bioinformatics study show a complete correlation of the clinical severity with the predicted damage this mutation causes to the pro-thrombin protein. The mutation Cys90Ser disrupts an important loop in the protein structure. In a report from south India, out of five different mutations identified, four were missense and one was in-frame deletion. None of these mutations were predicted to produce truncated or no protein at all. This was in agreement with another study which hypothesizes that a complete absence of prothrombin is incompatible with life, as has been demonstrated in a knockout mice model. Conclusion: The novel c.G269C homozygous mutation caused a Cysteine to Serine substitution at residue 90 of the primary prothrombin protein. We propose to refer this mutation as Prothrombin Mumbai.

Patient Id No.	Exon/intron	Mutation	Polymorphism	Comments
1	Exon 4	Arg 174 Stop		Homozygous
	IVS 1	–	A-246G	Homozygous
2	IVS 1	–	A-246G, A-61T	Heterozygous
	Exon 3	Glutamine 85 Stop		Homozygous
3	IVS 1	–	A-246G	Homozygous
	Exon 5	Glutamine 230 Arg		Homozygous
	Exon 8	–	Pro331Pro	Homozygous
4	IVS 1	–	A-246G	Homozygous
	Exon 14	Val650 Ileu		
		Arg 681 Glutamine		
5	Exon 6	Arg 260 Hist		Homozygous
	Exon 12		Pro 564 Leu	
6	Exon 2	Ser19 Pro		Homozygous

Abstract 021

Mutations in Severe Factor XIII Deficiency Cases

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Background Coagulation factor XIII (FXIII) belongs to a family of transglutaminases and is the last enzyme to be activated in the coagulation pathway. It cross links α- and γ-fibrin chains to form a stronger clot that is resistant to fibrinolysis. Congenital factor XIII deficiency is a rare autosomal recessive disorder affecting 1 in 1–5 million individuals the prevalence of which is higher in countries where consanguineous marriages are common. Congenital Factor XIII deficiency is a serious bleeding diathesis the common symptoms being bleeding from the umbilical stump, prolonged bleeding post injury, intra cranial bleed and spontaneous abortions in women etc. FXIII deficiency is usually attributed to mutations in the Factor XIII A gene which is located on chromosome 6 and has 15 exons. Detection of these mutations is very important to study the molecular basis of FXIII deficiency and also for genetic diagnosis in families too. *Aim* To characterize the molecular background of FXIII deficiency in India. *Methodology* We have studied the mutations in six FXIII deficient patients who were so diagnosed on the basis of their clinical history normal screening coagulation values and most important of all the traditional urea clot solubility assay. Genomic DNA of these patients was extracted from 9 ml citrated blood samples by the standard phenol chloroform method. Genomic DNA was screened for FXIII A gene defects by PCR and direct sequencing strategy. Mutations were identified in all these patients. *Results* Following mutations were identified in all the six patients. *Conclusion* We have been able to identify the causative mutations in the FXIII A gene of six FXIII deficient patients, the highlighted ones amongst them being novel whereas the others were reported earlier. High heterogeneity in mutational profile has been observed in the present study. The data obtained would enable us to give an accurate diagnosis in all affected families by direct mutation analysis and also assist in establishing a National Mutation Database.

Abstract 022

A Female Haemophilia with A Missense Mutation (p.G420S) in A1 Domain of Factor VIII

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Background: Haemophilia being an X-linked disorder, does not directly affect females, although they can be carriers of this disorder. A female haemophilic (400 cases worldwide) is possible only when a carrier female marries a haemophilic male, a very remote combination. *Aim of the Study* To identify the causative mutation in a female hae-mophilia. *Methodology* We analyzed a female who presented with a history of swelling in the joints and history of severe bleeding diathesis. She was born of consanguineous parentage between a carrier mother and haemophilic father with a moderate degree of severity (FVIII: C 5.5%; VWF: Ag 120%) of haemophilia A (HA). She belongs to a family with an extensive history of consanguinity and incidences of moderate HA. The entire F8 gene was screened using multiplex PCRs and Conformation Sensitive Gel electrophoresis (CSGE). Bands showing altered mobility were sequenced to confirm the mutation. *Results* A mutation in the exon 9 (c.G1315A, p.G420S) was found in the homozygous state in this female, the same mutation was found in her male siblings thus confirming the mutation. The mutation results in change of polarity. The substitution leads to a polarity change between glycine and serine in the protein coil. The hydrogen side-chain of glycine generally contributes to the flexibility of the protein structure, whereas serine seems to induce the formation of the helix by its larger side-chain, thus influencing the secondary structure of the protein. *Conclusion* This is the first case of female with HA from India. In such cases, the need for genetic diagnosis is not required as all the progeny of such females would lead to carriers daughters or affected sons.

Abstract 023

Bone Marrow Changes in Symptomatic HIV Positive Patients with Special Mention of HIV Induced HLH and Anemia of Critically Ill

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Background Human immunodeficiency virus (HIV) has diverse clinical and pathological manifestation. Hematological manifestations are a major problem and it can be anemia, bicytopenia or pancytopenia. Causes of anemia can be vitamin B_{12} deficiency due to HIV itself or HAART therapy, nutritional deficiency of iron or functional iron deficiency secondary to chronic infection (anemia of chronic disease). A new entity described in AIDS cases is anemia of critically ill when the cytokines (IL-1, 6, TNF and TGF-β) block the release of iron from the macrophages to erythroid precursors. Various infective agents like Kala-azar also lead to pancytopenia. *Aim of the Study* To study the bone marrow findings in symptomatic HIV patients and correlate with clinical features. *Methodology* Thirty-eight HIV patients diagnosed at a tertiary care hospital between January 2008 and August 2010 were enrolled in this study. Only those confirmed HIV cases with clinical symptoms were included in this study. Clinical history including drug intake and other relevant histories were noted. Complete Hemogram and peripheral smear was examined. Bone marrow aspiration and biopsy were examined thoroughly. If required serum iron studies, triglycerides, fibrinogen and vitamin B_{12} were estimated. Special stain including Ziehl Neelson, periodic acid fast was also performed on bone marrows if required along with usual stains. Bone marrow changes were correlated with clinical history, therapy and presence opportunistic infections. *Results* Among 38 symptomatic HIV positive patients, 23(60%) were male. The age group varied from 25 and 70 years with median age of 45 years. The clinical indication of marrow examinations were pyrexia of unknown origin 18/38, pancytopenia 12/38 and refractory anemia 6/38. Anemia and thrombocytopenia were noted in 30/38 cases and 11/38 cases respectively. Bone marrow was normocellular in 17/38 cases, hyper cellular marrow in 13/38 cases and hypocellular marrow in 2 cases, one case shows dry tap. Erythroid dyspoiesis is seen in four cases. Megaloblastic changes seen in 13/38 cases with serum B_{12} being reduced in 10 cases. Anemia of critically ill with microcytic hypochromic RBCs and increased iron in reticulum cells of bone marrow with no iron granules in erythroid precursors was diagnosed in four cases with severe secondary infection. Workup in these cases revealed low serum iron and TIBC and raised ferritin levels. Eosinophilia was seen in six and hypolobated megakaryocytes seen in three cases. Hemophagocytosis was seen in seven cases of which two had significant hemophagocytosis of more than 3%. On further workup, these two cases had elevated triglycerides and ferritin and reduced fibrinogen level and was diagnosed as HLH as per 2004 criteria. Both were treated with HAART, ATT antibiotics with steroid being added as immunosuppresor for HLH. They responded to treatment within 2 months and the hemophagocytosis and pancytopenia settled. Kala-azar was diagnosed on bone marrow in two cases with PUO. Increased histiocytes and plasma cells (>5%) were seen in 8 and 10 cases respectively. Epitheloid granulomas were seen in four cases and ill formed granulomas seen in two cases which two shows acid fast bacilli in ZN stain. Gelatinous degeneration was seen in four patients, being severe in a patient on HAART for 6 years who had developed extra nodal paravertebral lymphoma recently. Conclusion: Bone marrow aspiration and trephine biopsy remains indispensable for evaluation of symptomatic HIV patients. Treatable causes of pancytopenia/anemia like tuberculosis, Kala-Azar, B12 deficiency, anemia of critically ill, HLH etc. can picked up early, and thus morbidity and mortality reduced in these patients.

Abstract 024

A Case of Adult T Cell Leukemia/Lymphoma (ATLL): Autopsy Revelation and Clinicopathological Correlation

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Introduction Adult T cell leukemia/lymphoma (ATLL) is a rare peripheral T cell neoplasm which shows rapid deterioration and can be fatal within 1–12 months. It is rare in India with only a few case reports. *Case Report* Our case was a 56-year-old male presented with asymptomatic leucocytosis of 40,000/cmm for 1 month. At presentation his hemoglobin was 14.8 gm% with no hepatosplenomegaly, lymphadenopathy, jaundice or skin lesions. He was under OPD management with a PBS diagnosis of leucocytosis with monocytosis. A few days later he developed moderate grade fever, mucoid cough with dyspnoea and orthopnoea. A diagnosis of Swine flu (H1N1) was considered as Pune was having an outbreak of the disease during the period. However, in the meanwhile the hematopathologist examined the peripheral smear which showed a predominance of atypical lymphomonocytoid cells with convulated and cerebriform nuclei. These cells were NSE positive (both dot and diffuse). In view of this bone marrow aspirate and flow cytometry was advised. The investigation for H1N1 flu was negative. Bone marrow aspirate showed similar atypical lymphomonocytoid cells. The flow cytometry revealed cells to be CD2, CD3, CD4, CD5 positive and negative for CD7, CD13 and CD33. Presence of CD4 and absence of CD8 suggested monoclonality, and therefore the possibility of ATLL was considered. Monoclonality was determined by performing PCR for TCR-γ gene rearrangement. Meanwhile the patient's condition deteriorated and he was found to have fine crepts over left infrascapular and infraaxillary regions, diagnosed as bilateral interstitial pneumonia, managed with antibiotics and ventilatory support. Chemotherapy consisting of cyclophophamide, vincristine and dexamethasone was started. However the patient's condition worsened and he succumbed to his illness. The clinical diagnosis of ATLL with no other tissue involved was given. Autopsy conducted revealed bilateral lung involved, showing singly scattered atypical cells, These cells were large with convulated, polylobated and cerebriform nuclei. Similar cells were seen in the hilar lymphnode and spleen. Discussion The ATLL is a rare entity which can present with several clinical variants. WHO divides this into four variants acute, chronic, smoldering and lymphomatous. Of these, the chronic and smouldering forms are relatively indolent whereas the acute and lymphomatous forms are more aggressive. Thus, our patient fits in the acute variant of ATLL, which deteriorates very fast with a survival of only 2 weeks to 1 year. This entity is so rare that the index of suspicion is low and the diagnosis is generally delayed, leading to increased morbidity and mortality. Conclusion: ATLL is rare in India and in the absence of classic clinical presentation, the diagnosis can be missed. Early diagnosis based on clinical and pathological parameters and knowledge of prognostic factors is important for early diagnosis and treatment of these cases.

Abstract 025

A Study of Immune Response Gene Polymorphisms in Inhibitor Positive Haemophilia A Patients

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Background The development of alloantibodies or 'FVIII inhibitors' to infused FVIII, seen in up to 25–30% severe and ~5% mild or moderate haemophilia A patients, is perhaps the most serious complication of FVIII replacement therapy. It leads to a considerable increase in mortality and cost of management of bleeds among these patients. In developing countries such as India, where resources are

limited, an increased incidence of post-operative inhibitor development has been reported, which usually proves disastrous during this critical time of wound healing. *Aim of the Study* Why some haemophilia patients develop antibodies while others do not and whether it may be possible to predict their development are still major issues that need to be resolved. Various host genetic and environmental non-genetic risk factors have been implicated, but there is no data on the predisposing risk factors for the development of these antibodies in Indian haemophilia A patients. We have investigated haemophilia A inhibitor positive patients for various polymorphisms in immune response genes viz. IL1β, IL4, CTLA-4 and TNFA, shown to influence antibody production in autoimmune disorders, with a view to finding a potential marker for the differential immune response to FVIII replacement therapy Methodology: The IL1β rs1143634 C/T *Taq*I SNP, the IL4 rs2243250 C/T promoter SNP; as well as the CTLA-4 rs5742909 C/T *Mse*I promoter and rs231775 A/G CDS1 polymorphisms, have been analyzed in over 30 inhibitor positive haemophilia A patients and 30 control inhibitor negative haemophilia A patients by the PCR–RFLP technique. Six TNFA SNPs, rs1800629 G/A, rs361525 A/G, rs1800630 C/A, rs4248158 C/T, rs1799724 C/T, and rs3093662 G/A have also been investigated in these patients by DNA sequencing. Cost-effective and quicker allele-specific PCRs have been developed to analyze the TNFA rs1799724 C/T and rs3093662 G/A SNPs. The results were analysed by Fisher's exact test for statistical significance. *Results* The C/T heterozygote genotype of the TNFA rs1799724 C/T polymorphism was found to be significantly higher in inhibitor positive patients (OR 6.136, *P* 0.0221, 95% CI 1.204–31.268). The other cytokine related gene polymorphisms showed no strong association. All these results are in contrast to the association of polymorphisms with inhibitors in other populations. *Conclusion* Further study into the association of these immune response gene polymorphisms with inhibitor formation, in a larger cohort of patients, as well as their association with other genetic risk factors of inhibitor development is required. This could provide useful insights into the immune response to FVIII in inhibitor positive haemophilia A patients, and possibly influence the timely prediction and prevention or treatment of FVIII antibodies.

Abstract 026

The Detection of 'Functional' FVIII Inhibitors by a Sensitive and Specific Chromogenic Assay

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Development of FVIII alloantibodies in patients with congenital Haemophilia A is perhaps the most serious complication of Factor VIII (FVIII) replacement therapy, which leads to increased mortality and cost of management of bleeds among these patients. Up to 25–30% of severe Haemophilia A patients and ∼5% of mild or moderate Haemophilia A patients develop "FVIII inhibitors" against the infused FVIII. Rarely, individuals with no bleeding history can also develop FVIII autoantibodies, a serious and potentially life-threatening bleeding disorder called acquired haemophilia. *Aim of the Study* The Bethesda assay has been the classical laboratory assay for the quantification of inhibitory FVIII antibodies since first published by Kasper et al. in 1975. Various modifications to this assay as well as new techniques like ELISA for the detection of FVIII inhibitors have been reported. However, the quantitation of FVIII antibodies still involves many important unresolved issues such as inter-assay variability, lack of standardization of the lower limit of inhibitor detection and the definition of a negative antibody titre, as well as interference by non-neutralizing antibodies. Hence, methods to detect very low-titre FVIII inhibitors on the rise without interference from non-neutralizing antibodies (lupus anticoagulants and other interfering antibodies that can co-exist with FVIII antibodies) are still being explored. *Methodology* We report here a sensitive and specific chromogenic assay, using the S-2765 substrate and Coamatic FVIII reagents (Chromogenix), which detects only functional FVIII inhibitors. We tested four FVIII:C inhibitor positive patients; which included three congenital haemophilia A patients and one acquired haemophilia patient. *Results* The assay was found to be sensitive up to a titre of 0.1 BU/ml FVIII:C inhibitor which is much lower than that detected by the conventional Bethesda assay (0.5 BU/ml). The assay was also found to be specific, without interference from lupus anticoagulant antibodies. The assay is much quicker and not more expensive than the Bethesda assay. Conclusion: Detection of very low FVIII titres earlier has specific advantages, in that, the therapeutic management of these patients can be modified before the FVIII inhibitor titre increases and has disastrous consequences. Also the assay could be very valuable as a screening technique prior to surgery here in India, since there is a high prevalence of post-operative FVIII inhibitor development in haemophilia A patients in this country.

Abstract 027

An Efficient and Affordable Epidemiological Sensor for Haemoglobin

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The existing technique for detection of haemoglobin (Hb) is to some extent biased towards laboratory based measurements rather than epidemiological studies for a large population. The high cost, time and trained manpower involved in the conventional methods (e.g. automated cell counting) often make such procedures unsuitable for guessing the population distribution of important parameters like haemoglobin and so needs a most suitable and economical method of estimation. Here a simple, cost effective visual test to determine blood haemoglobin concentration using 2, 6 dichlorophenolindophenol (DCIP) has been described. *Aim of the Study* To develop a new cost effective (without costly machine, well equipped laboratory and well trained technical person), simple, and accurate method for estimating haemoglobin concentration from a drop of blood. *Methodology* Blood of various haemoglobin concentrations from different individuals first added to DCIP solution. After certain time, the final reaction mixture takes different colours depending on the haemoglobin concentration. Colour shifts from blue to brown to red as the Hb concentration changes from low to high. Based on that different colour, we develop a colour strip as the reference colour scale for naked eye estimation of Hb. *Result* Colour strip has been developed by definite concentration of Hb samples. Unknown blood samples treated with DCIP solution produce a definite colour depending on their Hb content. Estimation of Hb content of the unknown samples can then easily be done comparing with the reference colour strip. *Conclusion* This method shows high level of accuracy and high precession though very cost effective compared to WHO recommended colour scale based rapid Hb test for Hb screening of a large population. Sensors yielding high throughput population data of basic clinical parameters like haemoglobin may thus significantly help in efficient and large scale epidemiological survey of population health. This in turn, may serve as a reliable economic index of growth and development of a population niche.

Abstract 028

Platelet Activation versus Aggregation: Critical Response to Gold Nanoparticle

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Background Gold nanoparticles are potential candidates for drug delivery, though recently it was found that it has a proaggregatory role on platelet. So basic understanding of this adverse response is very important in nano-drug delivery context. *Objective* This study was undertaken to investigate the broad spectrum of platelet gold nanoparticle interaction, mainly focus on (a) possible mechanism of gold nanoparticle induced platelet activation and aggregation, (b) how platelet responds to gold nanoparticles, in circulation or when adhered to a vascular bed. (c) Lastly whether there is any critical gold nanoparticle size where such response is more acute. *Methods and Results* Size variation of gold Nanoparticles were prepared by variation of citrate concentration. Gold nanoparticle of smaller size ($\sim$20 nm) induces platelets activation more potentially, which may or may not lead to aggregation. The aggregation as well as dense granule release is possible in some threshold pre activated conditions (presence of vascular bed analog with frictional shearing force or critical concentration of ADP). Without pre-activation, gold nanoparticle can only slightly increase the CD-62 P and tyrosine phosphorylation level whereas GpIIbIIIa expression either remain unaltered, slightly increased, or most of the cases decreased. In presence of Platelet antagonists like or apyrase and fibrinogen receptor blocker (R-G-D-S) gold nanoparticles nanoparticle cannot induce aggregation, even in pre-activated platelets. Conclusion: Human platelets show bi-stable behaviour with respect to variation of agonist like ADP. It is observed that nanoparticle induced pro-thrombotic effect is most conspicuous at the bifurcation point between a deggregatory, aggregatory phase. The transition point may serve as a functional marker of the platelets. Gold nanoparticles always activate platelets. Notably, this activation does not always result in aggregation. Aggregation only happened when there is an existence of threshold level of pre-activation. Platelets differentially respond to gold nanoparticles in adhere and suspended condition which is again explained in terms of different levels of preactivation in such conditions. The smaller size of gold nanoparticles ($\sim$20 nm) induces higher platelet activation and aggregation. Lastly platelet granule release is the probable cause gold nanoparticle induced platelet activation.

Keywords Gold nanoparticle, Granule release, Cone and plate, Activation, Aggregation

Abstract 029

Novel Conjugation of Nanoscale Materials: Potent Antiplatelet Drugs

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Background Metallic nanoparticles are exploited in various ways in nano-medicine, e.g., in treatment of cancer, HIV, or in for diagnostic purposes e.g. in making efficient MRI contrast agents. A hurdle in application of many nanomaterials through blood capillary is that they are reported to have pro-aggregatory effects on platelets. Synthesis of any nanomaterials lacking such pro-aggregatory effect will be beneficial in the general nanomedicine context. In addition, if the novel material shows any anti-platelet effect, a new class of drug design is envisaged. *Objective* The objective of the present work is to design novel nano-conjugates, showing anti-platelet effect. *Materials and Method*s Citric acid conjugated iron oxide nanoparticle (FeNP), starch coated (FeNP), citrated gold nanoparticle (AuNP), aspirin conjugated (AuNP) are synthesised by wet chemical methods. Platelet aggregation was measured by Chronolog aggregometer. The release of platelet granules in response to such nanoparticles were measured from surface CD-62 P expression using flow-cytometry, dense granule ATP release using Chronolog Lumi aggregometry. Immunoblot study of whole cell tyrosine phosphorylation indicates the intracellular signalling status. *Result* Citric acid coated (FeNP) shows anti-platelet effect. Though, citric acid is known to have a mild anti-platelet effect, in presence of (FeNP) accentuates this platelet inhibitory property. Interestingly, starch coated iron (FeNP) does not show any anti-platelet effect implying that it is enhanced entry of citrate in presence of nanoparticle conjugation, that induces this effect. Similar nano-formulation is shown to enhance the efficacy of aspirin to act as an inhibitor of the COX-II pathway. *Conclusion* (i) Nanoparticle conjugation can be designed in such a way that they have as anti-platelet drugs. (ii) The efficacy of conventional antiplatelet drugs can be improved by appropriate nanoformulation. (iii) The nanosurface can also be tuned to induce pro-aggregatory effects, such effects being beneficial for patients of hemorrhagic diseases.

Keywords Citric acid conjugated iron oxide nanoparticle (FeNP in C–T buffer), Starch coated iron oxide nanoparticle (starch coated FeNP), Citrated gold nanoparticle (AuNP), Aspirin conjugated gold-nanoparticle (ASA conjugated AuNP), Antiplatelet effect, Proaggregation

Abstract 030

Gold-Drug Nanoplex: A Novel Additive Drug Concept for Multiple Myeloma Model

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Background Cell selective responses of bare gold nanoparticles have been reported earlier. Vincristine Sulfate, salt of an alkaloid often used clinically in multiple myeloma. The mechanisms of action of this drug remain under investigation and so far known as its relation to the inhibition of microtubule formation in mitotic spindle, resulting in an arrest of dividing cells at the metaphase stage. The advantage of this drug that made it very acceptable is vincristine does not penetrate well into the cerebrospinal fluid. *Aims and Objectives* The recent principles of chemotherapy based on the application of multi-drug simultaneously. But, each of them has a unique toxicity and mechanism of action to enhance the therapeutic activity. But it is rarely possible to achieve equally good results without additive toxicity. We have tried to develop a novel Gold-drug nanoplex to achieve the better therapeutic measure and lesser toxic effect in multiple myeloma model system. *Method* Here we have conjugated the gold nanoparticles with

vincristine and that enhance the anticancer activity on a multiple myeloma cell line U266. *Result* In this report we have shown a novel characterization method based on the FTIR analysis and have shown that the extent of its therapeutic activity is directly depend on the extent of conjugation with the gold nanoparticles. The GNP-VS has shown the decrease survival rate than that of the VS and GNP itself. *Conclusion* The present work demonstrates that bare gold nanoparticles cause apoptosis in U266 human cells by arresting G0/G1 phase. When gold nanoparticles are conjugated with the myeloma drug vincristine, a known arrester of cell cycle, the effect is cumulative in nature. This confirms the suitability of the gold nanoparticles as a drug additive in multiple myeloma therapy. The altered cell cycle nature suggests the conjugate is acting on the phase of the lifecycle of the cancer cells as a better therapeutic agent without adding any additive cytotoxic agent like the other multidrug chemotherapeutic agents.

Abstract 031

Electrophoresis of Hemoglobin-Gold Nanoconjugate: The Simplest Primary Detection of Thalassemia

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Gold nanoparticles are well known for their various applications in biomedical science and therapeutics. We have settled up an electrophoresis technique by which one can very easily detect different types of protein migration pattern. Again, perturbation of protein electrophoretic profiles with nano-conjugation can have notable applications. The resolution of electrophoretic bands alter when proteins are conjugated with a smaller stoichiometry of nanoparticles, and a higher resolution of bands may be have important implication in some protein based diagnostic problems. In case of the nano-perturbation technique, the higher resolution of HbE separated under optimal nano-conjugation may be useful in distinguishing the E band from the A2 band, the ambiguity being one of the typical diagnostic problems encountered in carrier detection of thalassemia, HbE, disease being often miss-diagnosed as thalassemia carrier. Furthermore when the samples were run with other nanoform (Gold nanorod, GNR) GNR (prior incubation of 10 min was done), it was seen that the Eβ samples showed a characteristics migration pathway that was different from the other samples. Thus the technique will thus help to comprehensively detect different types of thalassemic patients.

Abstract 032

MPO Negative M3 AML: A Rarity

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Acute promyelocytic leukaemia (APL)/M3 AML is rare leukaemia comprising of 5–8% of AMLs. It affects mainly middle aged individuals coagulopathy is the main clinical abnormality leading to death of 3% of patients before initiation of therapy. Of the two variants namely typical and microgranular, the latter frequently presents with very high leucocyte count and a rapid doubling time. These microgranular promyelocytes though do not show granules on light microscopy are strongly positive for MPO both on cytochemistry and flow cytometry. We report a case of 31 years old serving soldier presenting with fever of short duration with mildly raised total leucocyte count and mild thrombocytopenia. Incidentally he was found to have abnormal hypogranular promyelocytes which on cytochemistry were MPO negative. Karyotyping revealed AML with t(15;17)(q22;q12). Flow cytometry showed absence of HLA-DR, CD34 expression and negative cytoplasmic MPO. Promyelocytes of APL, irrespective of type of variant, are known to be strongly MPO positive with both cytochemistry and flow cytometry. But this case proved to be an exception; a ray.

Abstract 033

Alteration in Bone Marrow Cellular Phenotype and Stromal Microenvironmental Association in Leukemia

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Leukemia is a heterogeneous disorder of bone marrow (BM) failure syndrome where normal hematopoiesis gets altered due to transformation of either the normal hematopoietic cell or the hematopoietic microenvironment or both. The existence of "leukemic stem cells" and their possible role in leukemogenesis have only recently been identified and it has changed the perspective with regard to new approaches for treating the disease. However the relationship between leukemic stem cells (LSCs) and leukemogenesis requires further investigation. In this present study, we have experimentally induced leukemia in mice by means of N–N′ Ethylnitrosourea (ENU) to investigate the alterations in normal bone marrow cellular phenotype and associated changes in the stromal hematopoietic microenvironment under the event of leukemic disease progression. We have identified a significant decrease in the normal HSC phenotype in terms of Sca1 and c-kit receptor expression and subsequent sharp increase in certain leukemic cell specific receptor expression like CD123, CXCR4 and CD44 in the leukemic bone marrow. The decreased HSC receptor (Sca1 and c-kit) expression profile with concurrent increase in the expression of leukemic cell specific receptors (CD123, CXCR4, CD44) by the bone marrow cells of leukemic mice may account for the possible transformation of the normal hematopoietic cells that is necessary for the disease initiation and progression. Some of these receptors like CXCR4 and CD44 are also known to play an important role in maintaining leukemic cells and their complex crosstalk with the surrounding stromal microenvironment. Thus up-regulation in CXCR4 and CD44 receptor expression essentially pointed towards the stroma dependent surveillance of the leukemic bone marrow cells in leukemia. Leukemic bone marrow cells documented a rapid generation of stromal feeder layer in culture. The rapid stroma generation further supported the fact that leukemic stromal microenvironment gets altered in possible ways to support leukemic cell generation and fueling leukemogenesis. The study presented here, has tried to hint at exploring new therapeutic strategies by not only identifying the expression profile of cell surface receptors unique to cells involved in leukemic progression but also targeting the specific components of the stromal microenvironment that would facilitate therapeutic management of the disease.

Abstract 034

Molecular Pathology of Severe von Willebrand Disease

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Background von Willebrand disease (VWD) is the most frequent inherited bleeding disorder caused by a quantitative and/or qualitative defect in von Willebrand factor (VWF), estimated to affect approximately 1% of the population in the western countries. Study at this institute suggests 10.8% of the patients with inherited bleeding disorder have VWD with most prevalent subtype as type 3. Genetic analysis is very laborious due to large gene size and distribution of mutation all over gene. The presence of highly homologous partial pseudogene copy (exon 23–34) in chromosome 22 is the major problem in molecular diagnosis. Diagnosis, genetic counseling, carrier and antenatal diagnosis play an important role in comprehensive management of these cases. *Aim of Study*: (1) Detection of mutations using various screening protocols i.e. CSGE, SSCP, PCR, RFLP to find out the efficacy and adopt a cost effective protocol. (2) To offer genetic diagnosis to the affected families by direct mutation detection techniques without the requirement of the family members or the index case. (3) To correlate the mutations with the phenotype and clinical attributes. (4) To find out the presence of founder mutations. *Methodology* In this study we adopted initial strategy for screening 11 CGA Arginine codons of the VWF gene in 100 unrelated severe VWD patients from all over India by PCR RFLP. All the PCR were standardized in our lab and exclusion of pseudogene was confirmed. We have designed 55 sets of primers for 52 exons, of which 30–32 exons to be screened for mutation by CSGE technique. Nine Multiplexes are standardized in our lab for 30 exons in VWF gene. *Results* Nonsense mutations could be detected in 17 (13 homozygous and 4 heterozygous) out of 100 severe VWD patients by PCR RFLP technique in 11 CGA codons. Four homozygous and one heterozygous mutations were detected in exon 31, in unrelated patients of Gaderia community from UP (North India). Intronic markers VNTR1, VNTR2 and VNTR3 are found to be similar in all these patients explains common mutation in this community. In exon 43, two homozygous mutations are found in two Muslim patients positive to VWF inhibitors. There are no reports of this mutation associated to inhibitor development in the literature. By CSGE technique we have detected two novel mutations (C2715X in Ex 50 and 1 Del CTCCCACG in Ex 40). In exon 28 we have detected 1 novel deletion mutation by direct sequencing. CONCLUSION: Direct mutation detection by RFLP analysis in hot spot regions is the most simple, less time consuming and cost effective method. This technique of PCR–RFLP is very efficient to detect mutation in von Willebrand disease which comprises of 52 exons and screening for mutation in type 3 VWD is difficult since mutations are not confined to the specific regions. Inhibitor to VWF is rare phenomenon, was seen in two patients with same mutation in exon 43. We could detect one nonsense mutation, one deletion mutation by CSGE method. 20% of our patients showed intronic change, which can be used as intragenic marker for the genetic diagnosis. Multiplexing and standardization of 3–4 exons is established and hence adopted a cost effective protocol for mutation detection in large gene like VWD. Antenatal diagnosis was offered successfully by intron 40 VNTR analyses in four families.

Abstract 035

Clinical Spectrum of Acquired von Willebrand Disease

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Background Acquired von Willebrand syndrome (AVWS) is a rare bleeding disorder that mimics the congenital form of von Willebrand disease (VWD) in terms of laboratory findings and clinical presentation. Unlike the congenital disease, AVWS usually occurs in individuals with no personal or family history of bleeding. AVWS occurs in association with a variety of underlying disorders, including lymphoproliferative disorders, autoimmune diseases and cardiovascular diseases etc. *Aim* To investigate clinical heterogeneity of VWD patients. *Methodology* We present five cases of AVWS from our center. Factor VIII activity was assessed by one stage assay and VWF: Ag by ELISA. The circulating autoantibodies to VWF were studied by both mixing studies using Platelet aggregation (RIPA) and ELISA method. ANA, ANF, anti-ds DNA was done, hormonal assay like T3, T4 and TSH is done. Anticardiolipin antibodies and LA was also studied in these patients. *Results*: Case 1: A 19-year-old girl came with history of gum bleed, malena and cervical swelling with high grade fever associated with chills and rigor. Laboratory investigations showed severe VWD, strongly positive to inhibitors to VWF. She was also positive for ANF, IgM and IgG anticardiolipin antibodies. CT scan findings were suggestive of granuloma in parietal region of the brain. Histopathology report on cervical lymph node biopsy was consistent with Kikuchi lymphadenitis. The patient died during treatment due to CNS bleed. Case 2: A 31-year-old female with history of menorrhagia, gum bleed with no family history was diagnosed to be severe VWD. She was positive for inhibitors to factor VIII and VWF. She was LA positive. Subsequently she presented with skin rash and joint pain, her ANA and anti-ds DNA antibody became strongly positive, these findings indicated that this patient had SLE associated with AVWS, which was ameliorated by corticosteroid treatment. Case 3: A 21-year-old female patient was referred for easy bruisability and history of menorrhagia. She was positive for anti-DNA antibodies with AIHA. These findings indicated that this patient had SLE associated with AVWS and AIHA. Treatment with corticosteroids improved SLE symptoms and corrected bleeding diathesis. Case 4: A 57-year-old female suffering from severe menorrhagia with a recent history of gum bleeds. She was also diagnosed with pituitary hypothyroidism with low levels of T3, T4 and TSH. She was treated for hypothyroidism with thyroxine tablets. On repeating the investigation her Factor VIII and VWF: Ag was found to be normal. She was diagnosed as case of acquired type 1 VWD with Hashimoto thyroiditis as the underlying cause of the syndrome. Case 5: 42 years male having severe bleeding symptoms since childhood with no family history, presented to us with hemarthrosis. He also suffered from seborrhic dermatitis and vitiligo. On mixing the normal pooled plasma with patient plasma the VWF: Ag was progressively reduced with time of incubation with disappearance of the multimeric bands. This patient was classified as a case of acquired type 3 VWD. *Conclusion* AVWS is a rare condition and can have diverse etiology. The condition is serious and has high morbidity and mortality. A high index of suspicion and good coagulation laboratory is needed to arrive at the correct diagnosis.

Abstract 036

Effects of Inorganic Arsenic on Cord blood and Bone Marrow Cell Population: An Investigation on Immune Toxicity

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Background Arsenic has been found in nature since antiquity. It has been recently reported that the exposure to even very low inorganic arsenic concentration through drinking water results in adverse health hazards. Though the WHO guideline value for arsenic in drinking water is 10 μg/l, some places in West Bengal and Bangladesh have a very high arsenic concentration in drinking water (>1,000 μg/l) which has become a great environmental as well as health concern. Arsenic is known to causes cancer of a variety of organs and is also associated with increased non-cancer diseases such as cardiovascular diseases, hypertrophy of liver and spleen, neurological disorders and diabetes mellitus. Surprisingly, the report concerning cord blood (CB) as well as bone marrow (BM) toxicity due to arsenic exposure has not been studied in details. However, it has been reported very recently that arsenic exposure can cause systemic immunodepression in several animals as well as in humans. Arsenic is able to cross the placental barrier. It has been reported that the concentration of arsenic in CB can be as high as in the blood of the exposed women. It is widely accepted that the developing immune system represents a particularly sensitive xenobiotic target. Thus, the exposure of arsenic may start very early in life, which poses a risk for low birth weight, preterm delivery and impaired fetal development. The hematopoietic system has the capacity to respond quickly to an increased demand for mature cells as a response to an external stimulus. Stem cells and mature hematopoietic cells circulate in the blood stream are usually more exposed to xenobiotics. This xenobiotics exposure can lead to cytotoxic effects on cell function either directly or in concert with immune mechanisms. Long term inorganic arsenic exposure through drinking water showed disturbed erythropoiesis, granulocytopenia and occasionally megaloblastic changes. *Aim of the Study* The aim of the present study is to elucidate the effect of in vitro inorganic arsenic toxicity in normal murine BM stem/progenitor cells population; their immune response and the effect on CB mononuclear cells. *Methodology* Murine BM cells were isolated using BM flushing techniques. Human CB cells were used as it is enriched in stem cell population. Both murine BM and human CB cells were cultured separately in presence and absence of arsenic (1 μM). The growth kinetics at different hours, cell viability and apoptosis pattern, proliferation index and colony forming assay being carried out in in vitro culture. The cell mediated immune parameters has been performed. The stem cell status was estimated by using flow cytometry technique. *Results* Inorganic arsenic is able to exert immunotoxic and immunodisruptive effects on BM and CB hematopoietic cells. An overall suppressive effect has been observed. *Conclusion* As arsenic crosses the placental barrier it can affect development too. Through our comparative study of arsenic toxicity in bone marrow and cord blood we clearly demonstrated the degree of damage in between these two hematopoietic cell populations by environmental arsenic exposure.

Abstract 037

Acute Leukemias of Ambiguous Lineage: Rare, Dubious, Difficult: Case Series

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Aim To analyse clinical, morphological and immunophenotypic profile of Acute Leukemias of ambiguous lineage. *Background* WHO refers to a rare entity of bilineage Acute Leukemias (AL) or biphenotypic AL (BAL) and groups it as 'AL of ambiguous lineage' along with AL with aberrant expression. BAL represents <5% cases of AL. Knowledge about BAL is limited in terms of clinical and biological presentation and with regard to outcome. More importantly, prognosis is poor compared with de novo AL. We present a series with varied presentation diagnosed as AL of ambiguous lineage in our tertiary care center. *Methodology* 24 cases of AL from a period of July 2009 to June 2010 were diagnosed on morphology according to French–American–British (FAB) classification. Detailed Flow cytometric immunophenotyping (FCI) using four-color flow cytometry (Becton–Dickinson FACS Calibur instrument; BD Biosciences) was performed on blast cell populations identified by CD45 versus side scatter properties using standard staining and analytical methods with leukocyte markers including surface CD5, CD7, CD14, CD10, CD19, CD22, CD13, CD33, CD34, CD45, CD117 and cytoplasmic CD3, CD 79a, MPO and nuclear TdT. *Results* Out of 24 AL cases, four cases (16%) were diagnosed as AL of ambiguous lineage of which two cases were diagnosed as BAL. One of these patients had presented de novo and another patient had relapsed after treatment for acute myeloid leukemia. One case was diagnosed as bilineage AL and also had relapsed to treatment for denovo AL. The fourth case was diagnosed as ALL with aberrant myeloid expression and responded poorly to therapy for ALL. When compared with Western data the incidence appears quite high for Indian population. Moreover, these patients showed higher incidence of CD34 antigen expression with higher rate of relapse with resistance to therapy and this carries a poor prognosis. *Discussion* In our center out of 24 cases of AL in 1 year we report four cases diagnosed as AML or ALL based on FAB. However, on FCI were diagnosed as BAL, Bilineage AL and ALL with aberrant myeloid expression which is around 16%—much higher than the Western literature. Limited studies are available for Indian population in this regard which prompted us to look into the biology as to why do they occur and study the biological characteristics in Indian population. Few studies have shown BCR-ABL in ALL and other aberrations which are not picked up by PCR presently. However, it is important for us to know that these mutations do exist and also to elucidate the nature of the neoplastic cells using multi-parameter analysis including morphological, molecular, cytochemical or immunological assays and perform risk stratification to make appropriate patient tailored decisions regarding chemotherapeutic regimes to achieve remission. At least patients who are not responding well, should be screened for ambiguous lineage using comprehensive FCI and molecular studies. The high incidence found prompts research centers to carry out 28 mutation studies since the prevalence is much higher and needs further studies. CONCLUSION: It is important to pick up cases of both BAL and bilineage AL. The prognosis of BAL patients is poor when compared with denovo acute myeloid leukemia or acute lymphoblastic leukemia and should be diagnosed at the earliest to achieve maximum.

Abstract 038

Heparin Induced Thrombocytopenia: Incidence and Laboratory Approach to Diagnosis in Indians

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Background The development of thrombocytopenia or a new thrombus in a patient receiving heparin necessitates careful assessment of Heparin induced thrombocytopenia (HIT), an antibody mediated complication of heparin therapy. As there can be other causes of thrombocytopenia one needs to distinguish heparin associated thrombocytopenia (HAT) from HIT because of the clinical implications. Western literature has reported the incidence of HIT to be 5–10% though the antigenic positivity has been reported in 40–50% cases of coronary bypass patients getting fractionated heparin. Only one work on HIT from India has been carried out in a sample pf 33 patients getting heparin where the incidence of HAT and

HIT was reported as 30 and 15% respectively. Is the incidence of HIT so high in India? And what would be the cost and time effective method for laboratory diagnosis formed the research question for this study. *Aims and Objectives* (1) To study the incidence of heparin associated antibodies resulting in HIT and Thrombosis (HIT-T) in patients undergoing coronary bypass surgery and open heart surgery as a result of unfractionated heparin. (2) To compare the efficacy of the two antigenic assays, i.e. rapid ID gel Microtyping system and ELISA test which pick up anti heparin PF 4 (anti H PF4) associated antibodies with the functional assays—the standard time-tested heparin induced platelet aggregation test (PAT) and the new rapid luminographic assay of heparin induced ATP release in the diagnosis of HIT. *Methodology* As primary objective was incidence of HIT in Indians the sample size was calculated as 122 as that would have enough power (80%) to give valid estimates at 95% confidence level about occurrence of HIT. Thus, 125 consecutive patients undergoing open heart surgery and getting fractionated heparin were included in the study. The platelets were recorded preoperatively and on post op day 1, 3, 5, 7 and 10. All relevant clinical details were noted. HIT was suspected in cases with >50% drop in platelet count from baseline. Heat inactivated plasma was stored at −70°C for ELISA for ant HPF4 antibodies and heparin induced platelet aggregation test (PAT) and luminographic test for ATP release. The ID gel test was carried out on fresh sera on same day. The PAT test was done by using reactive donor PRP, heparin 0.1 U/ml and patient plasma. And luminographic detection was done on same sample. Ratio of low and high dose heparin indicated by ATP release >5% was taken as positive. *Result* Thrombocytopenia was seen in 40/125 (25%) individuals. Clinical diagnosis was probable HIT (>50% drop in platelet count) in 11 (9%), unlikely/possible heparin-induced thrombocytopenia 29 (23%) and no thrombocytopenia in 85 (68%). Taking the presence of positivity of one functional test and one immunological assay result in these patients to be confirmatory of HIT, we confirmed 7 cases of HIT in 125 patients. Two of the patients with confirmed HIT succumbed on 3rd post op day, one due to development of sepsis, bleeding and shock and the other due to acute lung syndrome and pulmonary thromboembolism. Thus HIT-T developed in one of our patients. The antigenic assay (ELISA) was positive in 21/125 (17%) thus showing that the anti HPF4 antibody presence does not mean that patient has HIT. The sensitivity of the simple and rapid test based on ID gel test was found comparable to the functional assay. Compared with the ELISA the specificity was better with similar sensitivity. *Conclusion* The incidence rate of HIT 5.6% and HITT is 0.8% in Indians. As the thrombocytopenia can develop due to other causes, a patient should be clinically put into the category of definite/probable, possible and unlikely HIT. The ID-gel immunoassay for detection of anti-H-PF4 antibodies (IgG) is a rapid and easy to perform test which should be immediately done in all cases of suspected HIT. In cases of probable HIT it confirms the diagnosis, but in other categories it should still be combined with a functional assay to confirm HIT.

Abstract 039

Mast Cell Sarcoma of the Small Intestine: A Case Report

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Background Mast cell sarcoma is a rare disease characterised by local proliferation of atypical mast cells, destructive growth and poor prognosis. There are only few cases of mast cell sarcoma reported in literature so far. Here we present a case of mast cell sarcoma arising in small intestine. *Aim* To describe a rare case of mast cell sarcoma of the small intestine. *Materials and Methods* Specimen of small intestinal resection was grossed, tissue sections were paraffin embedded and H and E stained slides were examined. Ancillary studies included immunophenotyping of paraffin sections and special stains (Toluidine blue for metachromatic granules). *Results* The tumour presented in 69 year old male with abdominal pain and CT findings showed circumscribed growth in the jejunoileal region. Grossly, the specimen consisted of 15 cm long segment of small intestine which showed a constriction on the external surface. Cut surface showed a firm, light brown, homogenous, circumferential tumour measuring 4.5 × 4 × 1.5 cm. The tumour appeared to involve the muscle coat. Histopathological examination showed focal ulceration of the mucosa and transmural infiltration of the wall by tumour, composed of diffuse infiltrate of large immature cells having round to oval, lobated pale staining nuclei and moderate amounts of eosinophilic to clear cytoplasm. Cytoplasm showed metachromatic staining with Toluidine blue. Immunohistochemistry showed that the tumour cells were positive for CD43, KP1, CD117 and mast cell tryptase and negative for MPO, VS38, CD30, CD23, CD21, S-100 and pan B and T cell markers. Bone marrow trephine biopsy and aspirate smears were negative. *Conclusion* Based on the above histopathological findings and ancillary studies a diagnosis of mast cell sarcoma of small intestine was made. Mast cell sarcoma of small intestine is a rare tumour, with only one other case being reported in the world literature, to the best of our knowledge.

Abstract 040

Diagnosis of T Cell Acute Lymphoblastic Leukemia/Lymphoma by Flow Cytometry: Our Experience

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The Lymphoblastic Leukemia/Lymphoma (T-ALL/T-LBL) is a neoplasm of lymphoblasts committed to the T-cell lineage; comprising about 15% of childhood ALL. It is more common in adolescents and younger children and usually with a male predominance. A series of eight cases of T-ALL/T-LBL is presented which shows wide variation in clinical presentation, morphology and Immunophenotypic features. Three of the cases showed interesting and unusual clinical features. Clinical details of the cases along with a complete laboratory work up and review of literature will be presented.

Abstract 041

Epidemiology of Childhood Malignancy from Eastern India

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Over 40,000 new pediatric cancers are estimated to be diagnosed annually. The proportion of child-hood cancers relative to all cancers reported by Indian cancer registries varied from 2.1 to 6.2%. It is a prospective hospital based follow-up study in our pediatric oncology clinic to delineate the epidemiology of childhood malignancy from eastern India to help to formulate the health policy in cancer related child health problem in future. The study includes all malignancies excluding brain and bone tumors in the children up to 12 years who attended our pediatric oncology clinic in the span of 4 years. Brain tumours and bone tumour are excluded because they are mainly dealt in other specialties. Total no of childhood malignancies were 157,

which corresponds to approximately 6% of all hospital admission to pediatric ward during that period. The affected boys outnumber 94 (59.8%) the girls 63 (40.10%) with a ratio of 1.5:1. Age wise distribution is seen as <1 year 4 (3.06%), (1–5) year: 65 (41.4%), (5–10) years: 64 (41%), >10 years: 24 (15.2%). Below 1 year, all patients had solid tumor. Hematological Malignancy contributes to 102 cases (65%) and Solid Tumor comprises to 55 cases (35%). Out of all hematological malignancy ALL stands prominent with 62 cases (61%) followed by NHL with 21 cases (20.5%). Among all cancers, ALL was most common malignancy comprising 40.02%, NHL 13.37%, HD 5.73%, AML −5.09%. In solid tumor group, Nephroblastoma was seen in 11 cases comprising (20%) and Neuroblastoma in eight cases comprising (14.5%) respectively in our study.

Abstract 042

A Three-Year Follow-Up of CML Patients on Imatinib

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Background Chronic myeloid leukemia is caused by clonal proliferation of hematopoietic cells carrying Philadelphia (Ph) chromosome leading to constitutively active BCR-ABL tyrosine Kinase. Imatinib is a specific inhibitor of BCR-ABL Tyrosine Kinase. The efficacy and response to treatment is gauged on the basis of hematologic, cytogenetic and molecular responses. *Aim of the Study* To study the disease pattern in CML patients who have completed at least 3 years of treatment, in tertiary Cancer Centre in India. *Methodology* An analysis of 100 patients of CML-CP started on Imatinib 3 years back was done. Periodic Cytogenetic analysis (by conventional Karyotyping or FISH test) and Quantitative analysis of BCR-ABL transcript by RT–PCR were done for treatment monitoring. *Results* 100 patients of CML-CP, started on Imatinib 400 mg 3 years back, were analyzed for disease pattern and course of treatment. A complete Hematologic response (CHR) was observed in 95% of patients on Imatinib 400 mg at 3 months. Two patients were intolerant to Imatinib of which one was shifted to Interferon-alpha and one patient was started on Dasatinib. Cytogenetic response was assessed in 77 patients. Of these, 51% achieved a complete cytogenetic response (CCyR) at 1 year. An additional 26% of patients demonstrated CCyR at 2 years. Other seven patients were continued on a higher dose of Imatinib, two achieved CCyR with Dasatinib and four patients did not undergo further cytogenetic analysis. Molecular response was assessed in 93 patients. Of these, 85 patients achieved major molecular response (MMR) during the study period. Amongst patients in MMR, 59% of patients achieved MMR at 18 months, 27% at 24 months and 14% at 36 months. Amongst 50 patients who achieved MMR at the optimal time frame (18 months) eight relapsed later. Six patients however, regained MMR with escalated doses of Imatinib, one was found to be Imatinib resistant on mutational analysis and one patient progressed. An update of the data will be presented in the conference. *Conclusion* A cytogenetic analysis should be done at 6 months and then 6 monthly there after till achievement of CCyR. A quantitative molecular analysis should be done at 3 monthly intervals till major molecular response has been achieved. Thereafter, the test should be done at 6 monthly intervals. These guidelines are formulated by European Leukemia Net, though the applicability in the Indian scenario is influenced by logistics. This study demonstrated a major molecular response in 93% of patients which is in accordance with international data. All the patients in this study are currently on a regular follow-up.

Abstract 043

Clinico-Hematologic Profile of Patients with Haemoglobin E Syndrome in a Tertiary Care Hospital

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Introduction Hemoglobin E (HbE) is one of the most prevalent hemoglobin variant, which is widely distributed in Southeast Asia. It is the commonest hemoglobin variant in India with a high prevalence (7–50%) in Northeastern region. The present study was conducted to assess the clinical and hematologic profile of these patients. *Materials and Methods* Twenty-seven cases of Hb E syndrome were included in the study who presented in the hospital between May 2005 to March 2009. Their age at onset, clinical symptoms (hepatosplenomegaly, gall stones, skeletal abnormalities), need of transfusion were recorded. Complete hemogram, serum iron studies along with Hb electrophoresis was carried out with HbF and HbA2 quantification. *Results* Out of the 27 patients, there were eleven males and sixteen females. Pallor was the presenting symptom in all cases. The average age at presentation was 23 years. Three patients had homozygous Hb E disease, five had HbE trait and fifteen cases were heterozygous for Hb E-Beta Thalassemia trait. Eighteen had no history of blood transfusions (BT), seven were on occasional transfusions, and two were on regular transfusions at diagnosis. The correlation of haematological profile with clinical profile, genotype and transfusion requirement will be discussed. *Conclusion* Clinical severity of E-beta thalassemia is variable ranging from mild to severe simulating homozygous β-thalassemia. The management of HbE-β-thalassemia is also similar to that of homozygous β-thalassemia. Early diagnosis and institution of therapy can prevent growth retardation and other complications in these patients.

Abstract 044

The Prevalence of Irregular Erythrocyte Antibodies Among Antenatal Women in Delhi: A Tertiary Care Hospital

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Background Universal screening of all antenatal women including Rh D positive pregnant women is highly debated and controversial. Most of the developed countries have guidelines for screening of all pregnant women for irregular erythrocyte antibodies (IEA). Drug Controller General India has given the guidelines for screening of all Rh D positive and negative women. However, they are not followed strictly. Limited literature is available on immunization rates in pregnant women (Rh D positive and Rh D negative) from India. *Aim of the Study* The present prospective study was carried out to detect the prevalence of irregular erythrocyte antibodies among multigravida women in Delhi, India. *Methodology* The present prospective study was carried out at Regional blood transfusion centre (RBTC), Lady Hardinge Medical college and associated Hospitals, New Delhi over a period of one and half year; from June 2008 to Dec 2009. 3,577 multigravida women were ABO and Rh D typed and screened for irregular erythrocyte antibodies (IEA), irrespective of their period of gestation. A commercially available three cell antigen panel (ID Diacell I, II, III; DiaMed ID Microtyping System) was used for the

antibody screening procedure, followed by antibody identification using (Diamed 11 cell diapanel) if required. Alloimmunized patients were reviewed for medical history, detailed obstetric history, history of hemolytic disease of newborn (HDN) among siblings and history of blood transfusions. *Results* We found the overall prevalence of irregular erythrocyte antibody (IEA) in pregnant women to be 1.25%, with Anti Rh D antibody contributing to 78.4% of total formed antibodies. A total of 51 antibodies were detected in 45 patients with dual antibodies in 6 women. There was a statistically significant difference between alloimmunization rates in Rh D negative versus Rh D positive group (10.7% vs. 0.125%; $P < 0.001$). The prevalence of alloantibodies in antenatal women with adverse obstetric history was significantly higher than those without adverse obstetric history (5.5% vs. 0.52%; $P < 0.001$). *Conclusion* Due to fragmented and non uniform standards of care in ANC, Anti D is still the most significant and prevalent cause of alloimmunization in pregnancy in Delhi's population. We need to focus on universal and standardized Anti D immunoprophylaxis implementation before moving on to universal IEA screening.

Abstract 045

Myelodysplastic Syndrome: Our Experience in Tata Main Hospital, Jamshedpur

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Background Myelodysplastic syndrome (MDS) represents a group of clonal haematological disorder characterized by progressive cytopenia reflecting defects in erythroid, myeloid and megakaryocytic maturation. The incidence of MDS is more in older age groups and frequent chromosomal abnormalities reported to be monosomy 5q- and 7q-. *Aim of the Study* To analyse clinical, haematological, histomorphological and cytogenetic changes in 84 cases of MDS seen in haematology clinic at Tata Main Hospital, Jamshedpur over a period of 4 years (January 2006–July 2010). *Methodology* Complete blood counts were performed in an automated cell counter. Bone marrow aspiration and trephine biopsy were done in all cases. Chromosomal analysis was done in 34 cases. Both FAB and WHO classification have been incorporated. *Results* 48 cases were male and 36 cases were female. The mean age at presentation was 55 years (range 15–82 years). A majority of patients presented with weakness (72%) only. Autoimmune manifestations in the form of joint pain were present in 18% of cases. Patients were symptomatic for a prolonged period before diagnosis could be reached (average 106.8 days). Ten patients had chromosomal abnormality. *Conclusion* A majority of patients had MDS—Refractory anaemia (52%) and MDS-RA with excess blasts (16%) at presentation. Out of ten patients having chromosomal abnormalities 8 had deletion of 5q and 2 had deletion of 7q. 12 patients were relatively young at presentation, less than 50 years of age. A majority of patients opted for symptomatic treatment only.

Abstract 046

Metachronous Presentation of Carcinoma Breast with Chronic Myeloproliferative Disorders

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Introduction Therapy related-myeloid neoplasms such as myelodysplasia syndrome (MDS) and acute myeloid leukemia are well known in patients of carcinoma breast who received chemotherapy or radiotherapy. However it is rare to have carcinoma breast developing in a background of chronic myeloproliferative disorder (CMPD) in the same patient. We present three cases of chronic myeloproliferative disorders, one of chronic myeloid leukemia (CML) and two of primary myelofibrosis, who developed carcinoma breast. Case history 1: A 57-year-old lady presented with a lump in right breast since 4 months. She also complained of dragging abdominal pain and an abdominal lump (massive splenomegaly) since 1 year. Her presenting blood counts (CBC) were Hb: 8.6 g/dl, platelet count 466×10^9/l, TLC: 16.8×10^9/l with neutrophilia. Bone marrow examination revealed hypercellularity with increased megakaryocytes showing dysmegakaryopoesis and micromeagkaryocytes. The provisional diagnosis of primary myelofibrosis-cellular phase was offered. JAK2 mutation study done on peripheral blood confirmed the diagnosis. The breast biopsy confirmed infiltrating duct carcinoma (IDC), grade III and was negative for ER, PR and Cerb2. She underwent the modified radical mastectomy. She also underwent splenectomy which revealed extramedullary hematopoiesis. One year later, she had recurrence of carcinoma breast with metastasis to supraclavicular lymph nodes. Her last CBC showed Hb 9.6 g/dl, TLC 22×10^9/l, platelets 836×10^9/l. She is lost to follow up. Case history 2: A 42-year-old female presented with weakness and abdominal lump. She had moderate splenomegaly and her presenting CBC: Hb 11.4 g/dl, TLC 62.4×10^9/l, platelet count 269×10^9/l. Her bone marrow was compatible with chronic myeloid leukemia-chronic phase (CML-CP). Cytogenetic study revealed presence of Philadelphia chromosome and confirmed CML. She started on Imatinib 400 mg once a day. She achieved major cytogenetic response. Five years later she presented with a lump in right breast. Infiltrating duct carcinoma grade II with ER, PR positive and cerb2 negative was demonstrated on biopsy. She received anthracycline based standard chemotherapy and underwent modified radical mastectomy. Four years later, she developed Infiltrating duct carcinoma, grade III and positive for cerb2 and negative for ER and PR in the left breast. She received paclitaxel based chemotherapy and underwent breast conservative surgery. Her CBC at last follow up is Hb 8.9 g/dl, TLC 4.2×10^9/l and platelets 107×10^9/l. Case history 3: A 65-year-old female presented with weakness and menorrhagia for 2 months. She had massive splenomegaly. Her presenting CBC revealed pancytopenia with Hb 3 g/dl, TLC 1.5×10^9/l and platelets 1.2×10^9/l. Hepatoportal doppler study was normal and did not reveal portal hypertension. Bone marrow biopsy showed hypercellularity with increased megakaryocytes and mild fibrosis. Cytogenetic study for Philadelphia chromosome, myelodysplasia related abnormalities and JAK-2 mutation were negative. She was started on thalidomide 10 mg alternate day. Four years later, she developed a lump in left breast. Biopsy revealed Infiltrating duct carcinoma, grade III and was positive for ER, PR and negative for cerbB2. She received standard chemotherapy and was posted for surgery. Discussion: Though occurrence of malignancy involving various organs has been reported, carcinoma breast in a patient with CMPD or followed by CMPD. This rare metachronous presentation of carcinoma breast with CMPD is highlighted in our case report.

Abstract 047

Role of Genetic Variants Influencing HbF Production in HbE-β Thalassaemia

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HbE (Codon 26(Glu→Lys)) is a common haemoglobin variant in India with prevalence of 7–50% in North eastern region and 1–2% in West Bengal. Interaction of Hb E with β-thalassemia show remarkable variability in the clinical expression, ranging from a mild form of thalassemia intermedia to transfusion-dependent conditions, clinically indistinguishable from homozygous β^0-thalassemia. Previous studies have shown that the clinical variability depends on the β and α globin genotypes and the genetic modifiers that are associated with increased foetal haemoglobin (HbF) synthesis. Pioneering work in this area has demonstrated that the C→T polymorphism at position −158 of the HBG2 promoter (rs7482144) represents a major quantitative trait locus (QTL) for the regulation of HbF level. More recent studies have shown that genetic elements outside of the β-globin gene cluster, namely, BCL11A and in HBS1L-MYB loci are also implicated in HbF regulation. The aim of our study was to evaluate the role of these three genetic factors in regulating HbF levels and phenotypes in patients with HbE-β thalassemia in Indian population. We studied 161 HbE-β thalassemia patients who had the same β-globin genotype, IVS1–5(G→C)/β^E with normal alpha globin genotype ($\alpha\alpha/\alpha\alpha$). Haematological parameters (CBC) were measured on an automated cell counter (LH 750, BC, USA). Haemoglobin F and A$_2$/E were quantitated using an automated high performance liquid chromatography system (VARIANT, BIO-RAD, USA). Genomic DNA was isolated from peripheral blood leukocytes by standard protocols and the mutations in the beta globin genes was detected by reverse dot blot and the alpha globin genotyping was done by a multiplex PCR that detects seven common mutations prevalent in our population. The analysis of SNPs at HBG2, BCL11A and HBS1L-MYB loci were studied by PCR–restriction fragment length polymorphism (PCR–RFLP) analysis. Data analysis was performed by SPSS software. The median age of the cohort was 13 years (1–54 years) with 111 males and 50 females. Steady state untransfused haematological data were available for 44 patients. The mean Hb, HbF and Hb A$_2$/E levels in these patients were 7.3 ± 1.8 g/dl, 29.85 ± 12.95% and 60 ± 12.4%, respectively. The allelic frequencies of different SNPs are tabulated (Table 1). We found that there was no significant correlation between total haemoglobin or relative or absolute amounts of HbF with the SNPs at the BCL11A (rs4671393), (rs11886868) ($P = 0.19$, $P = 0.8$), HBS1L-MYB (rs4895441) ($P = 0.4$) and HBG2 (7482144) ($P = 0.9$) loci. Lack of association of these factors with the phenotypes in beta thalassemia has been observed in a study conducted in β-thalassemia intermedia patients from France. Recent genome wide association analysis studies in Hemoglobin E/β^0 thalassemia showed that SNPs in β globin gene cluster have association with HbF levels. An interesting observation in this study was a positive correlation between total haemoglobin and the absolute amounts of HbF and HbE. The significance of correlation of Hb with HbE (g/dl) was higher when compared with absolute HbF ($r = 0.736$ vs. 0.49; $P = 0.000$ vs. 0.001). A comprehensive analysis of all known genetic elements that affect the expression of HbF and HbE will help us to plan our future experiments for identification of newer population specific genetic factors that play a role in the phenotypic diversity of HbE-β thalassemia in the Indian patients.

Table 1 Allelic frequency of different SNPs in HbE-β thalassemia

SNP	Chr	Gene	Alleles	Wild type	Allele-2	Sankaran et al. (2008)		Galanello et al. (2009)	
						Cooperative study of sickle cell disease	Brazil	Thal int	Thal major
rs4671393	2	BCL11A	A/G	A = 0.87	G = 0.13	0.27(A)	0.24(A)		
rs11886868	2	BCL11A	C/T	C = 0.61	T = 0.39	0.31(C)	0.39(C)	0.48(C)	0.21(C)
rs4895441	6	HBS1LMYB	A/G	A = 0.91	G = 0.09	0.10(G)	0.14(G)		
rs7482144	11	HBG1	G/A	G = 0.55	A = 0.45	0.07(A)			

Abstract 048

An Analysis of ABO Discrepancies in a Tertiary Care Hospital

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Introduction The ABO system is the most important of all blood group systems in transfusion practice. ABO grouping is a simple, accurate and precise procedure and to be considered valid, the results of cell grouping and serum grouping should agree. Correct typing of a donor or a patient is crucial in transfusion practice since the naturally occurring IgM anti-A and anti-B antibodies can readily activate complement which lead to acute hemolysis and death. *Materials and Methods* We analysed blood group discrepancies reported in our department between Jan' 2009 to June' 2010. *Results* 23 blood group discrepancies were detected in a total of 20,119 donors samples and 25,242 patients samples. The most common cause of these discrepancies was ABO subgroups. Low titer of anti-B in serum samples, presence of naturally occurring cold agglutinins in sera and rouleaux formation were other reasons in this regard. *Conclusion* It is important to recognize discrepant results and resolve them. Correct blood typing and labeling of an individual is essential to prevent ABO incompatibility.

Abstract 049

Predictor Marker for Transfusion Requirement in Hemoglobin E-β Thalassaemia Patients

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Background Hemoglobin E-beta thalassaemia is a common haemolytic anemia in South-East Asia. The patients show remarkable clinical heterogeneity—ranging from asymptomatic to severe transfusion dependance. There is no specific marker to predict the frequency of blood transfusion required by such patients. *Aim of the Study* To find a factor, like Hemoglobin F, that can predict the transfusion requirement. *Methodology* Fifty-eight patients of Hemoglobin E-β Thalassaemia visiting the Department of Hematology, NRS Medical College and Hospital underwent proper clinical evaluation. They were subjected to the following two tests: Complete Blood Count and High Performance Liquid Chromatography (HPLC). They were grouped into the following three groups according to their age of first blood transfusion: Group 1: age of first blood transfusion <2 years. Group 2: age of first blood transfusion ≥2 years. Group 3: no blood transfusion. *Results* Out of 58 patients, 34 were male and 22 female with a median age of 23 years (range 2.33–55). Group 1 had seven patients; two male and five female with a median age of 22 years (range 3–26). Majority had spleen size ≥6 cm and a mean transfusion interval of 1 unit every 45 days. Median levels of the following parameters were: Hemoglobin 6.5 gm% (range 3–8.2), Hemoglobin F 10.8% (range 7.6–19.8), Hemoglobin E 38.3% (range 20.8–77.8) and ratio of Hemoglobin E/Hemoglobin F 3.83 (range 1.93–6.38). Group 2 had 46 patients; 26 male and 20 female, with a median age of 24 years (range 3.8–43). Majority had spleen size <6 cm and a mean transfusion frequency of 1 unit every 75 days. The median levels of the following parameters were: Hemoglobin 7.3 gm% (range 2.6–11.8), Hemoglobin F 20.65% (range 12.5–48.1),

Hemoglobin E 54.96% (range 31.7–74.4) and ratio of Hemoglobin E/ Hemoglobin F 2.60 (range 0.97–6.2). Group 3 had five patients; three male and two female, with a median age of 8 years (range 4–55). Most had mild splenomegaly. The median levels of the following parameters were: Hemoglobin 10 gm% (range 8.5–12), Hemoglobin F 20.9% (range 15.8–39.3), Hemoglobin E 67.30% (range 48.80–70.20) and ratio of Hemoglobin E/Hemoglobin F 3.07 (range 1.24–3.23). Significant statistical differences in steady state Hemoglobin levels existed among group 1 vs. group 2 ($P < 0.001$) and group 2 vs. group 3 ($P < 0.05$). Hemoglobin F levels had significant difference among group 1 vs. group 2 ($P < 0.01$) and group 1 vs. group 3 ($P < 0.05$) but not between group 2 vs. group 3 ($P > 0.05$). Spleen size among group 1 vs. group 3 showed a significant statistical difference ($P < 0.01$) as well. No significant difference in Hemoglobin E and ratio of Hemoglobin E/Hemoglobin F were seen across the three groups. *Conclusion* Hemoglobin E-beta Thalassaemia patients who had first blood transfusion within 2 years of age had a spleen size ≥6 cm in majority of cases. They had lower levels of steady state Hemoglobin and low Hemoglobin F % by HPLC. Vis a vis, those having first transfusion after 2 years of age had spleen size <6 cm, higher steady state Hemoglobin levels and a higher % of Hemoglobin F. So Hemoglobin F, age of first blood transfusion and baseline Hemoglobin level are the predictors of severity and transfusion requirement in patients of Hemoglobin E-β Thalassaemia.

Abstract 050

Identification of Drepanocytes Using Retic VCS in Beckman Coulter LH 750

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Objectives The Coulter LH 750 system uses VCS technology which measures Volume, Conductivity and light Scatter on each cell in a hydrodynamically focused stream. This system also includes an investigation screen that gives statistical information (Mean and SD) of VCS about the main WBC population and also describes about the red cell VCS during reticulocyte analysis. The irregular shape of mature red cells and reticulocytes produce unpredictable light scatter information when subjected to a laser beam at angle 0–90°. Extending our previous study on detection of Target Cells, Ovalocyte, Elliptocytes Dacryocytes/schistocytes based on RPD, we reviewed the Drepanocytes based on these parameters. This was used as basis to study the mature red cells in the non-retic area and to correlate with the presence of Drepanocytes in smears. This population shows increase in both Conductivity and Scatter Mean and SD of red cells in Non-Retic area along with a characteristic (cut-like) scatterplot pattern. *Methods* A total of 139 samples (K2 EDTA) were processed in the reticulocyte mode on the Coulter LH750 analyser. 100 of these samples were having normal blood picture (No poikilocytosis), 31 samples were having Dacryocytes/schistocytes and 8 were having Drepanocytes on smear review. We compared the Non-retic VCS (Mean and SD) of samples with no poikilocytes to the samples having Drepanocytes and Dacryocytes/schistocytes. *Results* Both the Non-retic Conductivity and Scatter Mean and SD of the sample having Drepanocytes was higher with typical scatterplot pattern than the sample with no poikilocytes. We also compared the Non-Retic VCS of samples having Drepanocytes and Dacryocytes/schistocytes which shows only increase in Non-Retic Conductivity Mean. Using a cut-off in conductivity platform it was possible to discriminate samples that contained Drepanocytes from that with Dacryocytes/schistocytes with fair degree of specificity and sensitivity even though both the Scatterplots of Drepanocytes and dacryocytes/schistocytes exhibit identical (cut-like) pattern. *Conclusions* It is possible today with the instruments of the LH700 series from Beckman Coulter to create rules not only with the classical parameters MCHC, RDW, etc. but also with the reticulocyte Research Population Data and produce flags that may be will increase the detection of cases with these red cell abnormalities. This report adds on to our previous reports on other poikilocytes (Codocytes, Elliptocytes, Ovalocytes and Dacryocytes/ schistocytes) detection on automated cell counter LH 750.

Table 1

	Drepanocytes vs. normal red cells				
	ROC AUC	Sensitivity	Specificity	Cut-off	*P*-value
Nretconme	1.000	100	100	>68	0.0000
Nretconsd	0.980	100	95	>24.97	0.0001
Nretscme	0.989	100	93	>63	0.0001
Nretscsd	0.736	100	62.5	>16.76	0.02

Table 2

	Drepanocytes vs. dacryocytes/schistocytes				
	ROC AUC	Sensitivity	Specificity	Cut-off	*P*-value
Nretconme	0.853	67.7	87.5	>73	0.0001

Table 3

	Dacryocytes/schistocytes vs. normal red cells				
	ROC AUC	Sensitivity	Specificity	Cut-off	*P*-value
Nretscme	0.982	96.77	93	>63	0.0001
Nretscsd	0.908	90.3	85	>16	0.0001

Abstract 051

Increased *ABCG2* Expression Could be Responsible for Resistance to Imatinib Mesylate in Patients with Chronic Myeloid Leukemia

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Background Mutations in the *BCR-ABL* kinase domain is one of the most common mechanism of resistance to Imatinib mesylate, seen in 30–90% of patients with Chronic myeloid leukemia (CML). In addition, altered expression of the transporter genes, increased binding of Imatinib to alpha-1 acid glycoprotein or the activation of alternative anti-apoptotic pathways have also been considered as factors influencing Imatinib resistance in CML. *Aim of the Study* To evaluate expression levels of the efflux transporters (*ABCB1*, *ABCG2*) and the influx transporter (*hOCT1*) in CML patients who have not achieved Major Molecular Response (MMR) at/around 1 year after the start of

Imatinib (11–18 months) and to compare these levels in patients with and without *BCR-ABL* kinase domain mutations. *Methodology* At our center, International scale (IS) for expressing *BCR-ABL/ABL* ratio was set up in collaboration with Institute of Medical and Veterinary sciences, Adelaide. Quantitation of *BCR-ABL* transcript level was done by RQ-PCR using Taqman principle and the *BCR-ABL/ABL* ratio was expressed on the IS to determine the achievement of Major Molecular Response (MMR) at/around 1 year after the start of Imatinib. RNA samples at/around 1 year after the start of Imatinib were available for 120 patients, out of which 81 had not achieved MMR. These 81 samples were tested for the expression of *BCR-ABL* transcript by RQ-PCR and were also screened for *BCR-ABL* kinase domain mutations using Reverse transcriptase polymerase chain reaction (RT–PCR) amplification of the *BCR-ABL* kinase domain followed by direct sequencing. The mRNA expression levels of efflux transporters (*ABCB1, ABCG2*) and influx transporter (*hOCT1*) were measured in the same sample by RQ-PCR using *GAPDH* to normalize the expression levels of the same. *Results* Among patients who had not achieved MMR, 15 patients had *BCR-ABL/ABL* ratio between 0.1–1%, 18 had *BCR-ABL/ABL* ratio between 1–10% and 48 had *BCR-ABL/ABL* ratio >10%. In patients with *BCR-ABL/ABL* ratio 0.1–1%, there was significantly higher *hOCT1* expression (median 86.17 (38.79–149.2) vs. 29.48 (18.11–74.57) and 33.63 (2.82–127.3), $P = 0.017$) but no significant difference in *ABCG2* and *ABCB1* expression when compared to patients with *BCR-ABL/ABL* ratio 1–10% and >10%. Upon subgroup analysis comparing transporter expression in patients with *BCR-ABL/ABL* ratio >10% with and without mutation, there was significantly higher *ABCG2* expression in patients without *BCR-ABL* kinase domain mutation compared with patients who had mutations in the kinase domain (Table). *Conclusion* This result suggests that increased expression of *ABCG2* may be one of the mechanisms of resistance in patients who do not have *BCR-ABL* kinase domain mutations but have not achieved MMR. Future studies should be designed to compare the expression of these transporters at diagnosis (before the start of Imatinib) and also at the time of clinical resistance. This will help understand the influence of expression levels of these transporters in achieving cytogenetic or molecular response to increased doses of Imatinib.

BCR-ABL/ABL ratio on IS scale	*ABCB1/GAPDH* Median (range)	*ABCG2/GAPDH* Median (range)	*hOCT1/GAPDH* Median (range)
>10% With mutation	39.76 (5.33–178)	60.83 (22.68–242.7)	33.63 (4.29–137.4)
>10% Without mutation	29.57 (3.62–352.8)	127.6 (6.65–318.7)	46 (2.82–147.8)
P-value	NS	0.0054	NS

Abstract 052

Spectrum of Inherited Platelet Disorder in a Tertiary Care Centre

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Introduction Disorders of platelet function are characterized by highly variable mucocutaneous bleeding manifestation and excessive haemorrhage after surgical procedures or trauma. Platelet aggregation and secretion studies provide evidence for the defect. Patient with defects in platelet secretion and signal transduction are a heterogenous group. The major common characteristic in these patients are currently perceived, is an inability to release intracellular (dense) granule contents on activation PRP with agonist such as ADP, Epinephrin and collagen. In aggregation studies, the second wave of aggregation is blunted or absent. A small proportion of these patients have a deficiency of dense granules stores (Storage pool deficiency). In some patients the impaired secretion results from aberrations in the signal transduction events that end response such as aggregation and secretion. This study is to focus on this subset of patients, who are encountered more often than those with thrombasthenia or BSS. *Materials and Methods* The coagulation data of all patients who have been evaluated in the coagulation laboratory of CMC from Jan 2008 to Aug 2010 was received from records. Clinical history and laboratory data of patients with inherited platelet disorders suggestive of signal transduction and secretary defects was analysed in detail. Platelet aggregometry studies was done on Chrono-Log using platelet rich plasma with agonist like Ristocetin, ADP, Collagen, Epinephrine, Arachidonic acid TRA (Thromboxane receptor analogue), TRAP (Thrombin receptor activating peptide), and ATP release (Lumi Aggregometry). *Results* 25 patients were in this remarkably heterogenous group of platelet secretion/signal transduction defects which was classified as ADP receptor defect (2/26), Collagen receptor defect (1/26), Grey platelet syndrome (3/26), Storage pool defect (8/26), Signal transduction defect (11/26). Patients demographics includes Median age of 11(range 0.6 months to 47 years), mean age of onset was 3.3 years, male/female ratio was 1:0.8, consanguinity was elicited in 38.4%, mild to moderate bleeding history within the family was noticed in 33%, 33.3% of patients required transfusions. Most common symptoms were ecchymotic patches (65%), easy bruisability (30%), epistaxis (26%), followed by menorrhagia (19%), gum bleeds (23%) and post traumatic prolonged bleeding (20%). Abnormal laboratory data which includes mild thrombocytopenia (38,000–396,000), abnormal platelet morphology—giant platelets (38%), hypogranular platelets (19%), grey platelets (19%). Bleeding time was abnormal in (40%). Results of platelet aggregometry findings were listed in Table. *Conclusion* There is a definite need for concerned studies with a familial bleeding diathesis and abnormalities in agonist mediated platelet aggregation and secretion to unravel the aberrant pathways in these patients, There is also a need to standardize and to improve the diagnostic tests available, we have used a minimal diagnostic criteria to conclude these subset of patients who are most commonly encountered.

Platelet aggregometry studies

	ADP				AA			Collagen			TRAP			TRA			ATP release	
	Ab	Pri	Pri Rev.	Nor	Ab	Pri	Nor	Ab	Pri	Nor	Ab	Pri	Nor	Ab	Pri	Nor	Nor	Ab/Dec
Signalling defect (11)	–	–	10/11	–	4/11	1/11	6/11	–	1/11	10/11	3/11	3/11	3/11	2/11	8/11	–	11/11	
ADP receptor defect (2)	–	–	2/2		–	–	2/2	–	–	2/2	–	–	–	–	–	–	2/2	
Collagen receptor defect (1)	–	–	–	1/1	–	–	1/1	1/1	–	–								
Storage pool defect (8)	1/8	4/8	3/8	–	2/8	4/8	1/8				1/8	2/8	2/8	5/8	–	–		8/8
Grey Platelet syndrome (3)	–	–	–	3/3	–	–	3	–	–	3/3	2/3	–	1/3	2/3	1/3	–	3/3	

Ab Absent, *Pri* primary response, *Pri. Rev.* primary reversible response, *Nor* normal, *Ab/Dec* absent or decreased

Abstract 053

MTHFR Genetic Polymorphisms and Susceptibility to Acute Myeloid Leukemia in India

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Background Methylenetetrahydrofolate reductase (MTHFR) is an
essential enzyme in the metabolism of folate. The presence of poly-
morphisms that reduce the activity of MTHFR has been linked to the
multifactor process of development of acute leukemia. Studies on
MTHFR polymorphisms in acute leukemia have largely focused on
their protective role in acute lymphoblastic leukemia. Limited data is
reported in literature for acute myeloid leukemia (AML), and none for
the Indian patients. *Aim* To investigate the association between
MTHFR polymorphisms and the risk of acute myeloid leukaemia.
Methods Total genomic DNA was extracted from 63 patients of AML
(9 children and 54 adults) and 155 normal controls. MTHFR variant
alleles were determined by a PCR-restriction fragment length poly-
morphism assay and followed by *Hin*fI and *Mbo*II restriction
digestion. *Results* 1298AC variant was under-represented among the
patients when compared with normal controls (38.10% vs. 55.48%).
We found a statistically significant reduced risk of AML in individ-
uals with the 1298AC polymorphic variant as compared to 1298AA
when adjusted for age (OR = 0.606, adjusted OR = 0.426, 95%
CI = 0.310–1.185, adjusted 95% CI = 0.196–0.923, $P = 0.144$,
adjusted $P = 0.031$). C677T genotypes were not associated with the
risk of the disease. *Conclusion* Our findings demonstrate that the
MTHFR polymorphic variant 1298AC confers a protective effect,
thus modulating the risk of AML in the Indian population. In addition,
A1298C rather than C677T may be a more important genetic risk
modifier in leukaemogenesis in the Indian patients.

Abstract 054

Carbonyl Reductase-1 RNA Expression Influences In Vitro Daunorubicin Cytotoxicity and Intracellular Daunorubicinol Levels in Primary AML Cells

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Resistance to chemotherapeutic drugs, mainly cytarabine and Dau-
norubicin (Dnr) is one of the causes of treatment failure in AML.
Among other clinical and biological factors, overexpression of efflux
transporters (mainly ABCB1 and ABCG2) and Dnr biotransforming
enzymes (Carbonyl Reductase 1 (CBR1 and CBR3) have been shown
to be associated with Dnr resistance. CBR1 is the major enzyme
involved in the biotransformation of Dnr to daunorubicinol (DOL).
DOL is less potent than Dnr[1] and overexpression of CBR1 in K562
cell line has been shown to have increased resistance to Dnr[2]. Inter-
individual variation in Dnr metabolism may have potential implica-
tions on the toxicity and efficacy of the drug, making it critical to
understand the factors influencing this variability. The aim of the
present study was to investigate factors responsible for the inter-
individual variation in Dnr metabolism in AML patients. Bone mar-
row from 64 adult denovo AML patients (excluding AML-M3) at
diagnosis was included in this study. Total cellular RNA was
extracted using Tri Reagent and cDNA was synthesized. mRNA
expression levels of CBR1, ABCG2 and ABCB1 relative to house
keeping gene GAPDH was analyzed using Taqman based gene
expression assays. In vitro cytotoxicity was assessed using MTT cell
viability assay and IC50 was calculated. Patients were classified as

sensitive and resistant to Dnr based on the IC50 values <0.5 and
>0.5 μM respectively. Intracellular level of Dnr and its primary
metabolite DOL in primary AML cells incubated with 5 μM Dnr was
measured using HPLC coupled with fluorescence detector. There was
85 and 40 fold variation in the intracellular Dnr (17–1453 ng/
3×10^6cells) and DOL (1–40 ng/3×10^6cells) levels respectively.
IC50 of Dnr ranged from 0.01 to 3.15 μM. ABCB1 and ABCG2 and
CBR1 RNA expression showed 575, 990 and 660 fold variation
respectively. When we compared Dnr IC50 with RNA expression in
sensitive versus resistant patients, there was significantly higher
CBR1 expression in patients with Dnr IC50 >0.5 (median 0.923,
range 0.544–3.157) compared to those <0.5 μM (median 0.173, range
0.011–0.49, $P = 0.0148$) (Figure). CBR1 RNA expression was also
significantly higher in patients with intracellular DOL levels >10 ng
(median 19.9, range 10.08–40.22) compared to those with <10 ng
(median 6.79, range 2.76–9.84, $P = 0.0044$) (Figure) but no associ-
ation was seen with intracellular Dnr levels. mRNA expression of
ABCG2 and ABCB1 did not show any significant association with
Dnr IC50, intracellular Dnr or DOL. Functional assay of the ABCG2
and ABCB1 needs to be done to evaluate the role of these transporters
to better explain their influence on Dnr IC50 as well as intracellular
levels. Though CBR1 expression showed significant association with
Dnr IC50 and intracellular levels of DOL, overlap in CBR1 expres-
sion between groups limits its role in predicting drug resistance. This
is the first study to show the role of CBR1 in resistance to Dnr in
AML patients. To conclude, Dnr metabolism shows wide inter-indi-
vidual variation in AML and so are the genes involved in their
transport and metabolism and also that CBR1 is the major enzyme
involved in Dnr metabolism.
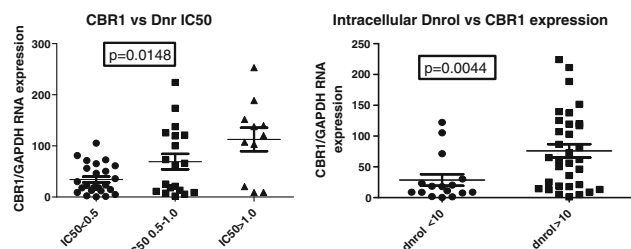
Abstract 055

Genotype–Phenotype Correlation of Hereditary Factor V Deficiency in India: A First Report

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Congenital deficiency of Factor (F)V is a rare (1:1,000,000) autoso-
mal recessive disorder caused by mutations in the F5 gene, in which
~70 disease causing mutations have been reported so far (HGMD[®]
database). We have investigated five unrelated Indian patients with
congenital FV deficiency. Their diagnosis was based on prolonged
prothrombin time, prolonged activated partial thromboplastin time,
normal thromboplastin time and FV coagulant (FV: C) activity
measured using a prothrombin time based one stage assay. All these
patients presented with severe disease (FV: C <1%). Genomic DNA
was screened for mutations in the F5 gene by a novel multiplex PCR
and conformation sensitive gel electrophoresis (CSGE) strategy. Four
novel disease causing mutations were identified in these patients.

They include a micro-deletion, a non-sense mutation and two missense mutations. A novel 2 bp deletion (c.3019_3020delGA X17) was identified in homozygous state in an 8-year-old female born of a consanguineous marriage. This patient had a severe clinical phenotype with a history of umbilical stump bleeding at birth, frequent gum bleeds and prolonged post-traumatic bleeds that frequently required fresh-frozen plasma (FFP) to control. This mutation predicts a frameshift from Glu1007 in the B domain, resulting in pre-mature termination of translation at codon 1024 of F5 and is consistent with severely reduced FV levels (FV: C <1%) in this patient. A novel homozygous c.2953C>A transversion in exon 13 of F5 gene, predicting a nonsense mutation p.Glu985X was identified in two patients. This mutation results in a stop codon in the B domain, resulting in a truncated FV in these patients. A lack of correlation between in vitro FV: C values and the clinical phenotype were noticed in these two patients. A mild-moderate phenotype characterized by easy bruisability and prolonged bleeding after dental extraction was observed in the first patient with p.Glu985X mutation, whose age at first clinical presentation was 9 years. However, the second patient, 1.5 years of age, had an earlier onset of severe bleeding symptoms (subgaleal haematoma at birth and a history of subcutaneous haematomas requiring intervention by FFP) in spite of having the identical p.Glu985X mutation. Although the mechanism of this phenotypic diversity is not clear, it would be of interest to determine the thrombin generating potential of both these patients as well as the co-inheritance of thrombophilic mutations. A p.Met203Arg missense mutation (c.608T>G) was identified in a 2-month-old male, presenting with intra-cranial haemorrhage and a history of umbilical stump bleed treated with FFP. The substitution at codon 203 of a hydrophobic (Met) residue by a basic (Arg) residue significantly impacts the secondary structure of the A1 domain and is likely to inhibit the interactions between the FV: A1 domain and factor Xa in this patient. A second novel missense mutation p.Leu2190Pro (c.6569T>C) was identified in homozygosity in a 5-year-old male with a history of muco-cutaneous bleedings. This mutation occurs at a highly conserved residue (FV: human, swine, murine) in exon 25 of F5 gene. Leu2190 occurs in an alpha-helix at the C-terminal domain (C2) of FV protein and is part of the soluble phosphatidylserine binding pockets (amino acids: 2037–2190 flanked by two tryptophan residues) which are known to be critical for FVa assembly on platelet membranes to form the "prothormbinase" complex. The substitution of this residue by proline, a known helix breaker, is likely to destabilize this region of FV and impair its assembly on platelets (FV: C <1%). In conclusion, we report here the identification of four novel mutations in FV gene and their molecular pathology presented. This is the first report describing mutations in this gene from India and the data contributes significantly to the mutation database of this condition as well as help in its genetic diagnoses.

Abstract 056

Analysis of Iron Regulatory Genes in Patients with Unexplained Iron Overload

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Hereditary hemochromatosis (HH) is the most common, identified, genetic disorder in the Caucasian population, however it is rare in the Indian population and very few reports exist in the literature. HH is characterised by iron overload, primarily in parenchymal cells resulting in tissue damage and liver cirrhosis, diabetes mellitus, arthropathy, cardiomyopathy, endocrine abnormalities and an increased risk of hepatocellular carcinoma. Both iron overload and iron deficiency anemia are under control of various genetic and environmental factors. Inappropriate production of hepcidin or a defective interaction with its cognate receptor ferroportin (SLC40A1) has been identified as the main pathophysiological mechanism in HH. Mutations in the Histone Family E1 protein (HFE) gene have been strongly implicated in HH. In addition, mutations in genes coding for hepcidin (HAMP), regulators of hepcidin like transferrin receptor 2 (TFR2) and haemojuvelin (HJV) and the iron efflux protein (SLC40A1) have also been shown to result in HH. In this study, we report the genetic analysis of these genes in eight patients with unexplained iron overload. They presented with typical hemochromatosis symptoms like hyperpigmented skin, hepatomegaly and cirrhosis. Two patients had a history of one or more family member with similar clinical profile. They had raised transferrin saturation (80 ± 35.9%), elevated serum ferritin 1241.7 ng/ml (542.8–2276), were negative for HBV and HCV infection and had a normal ceruloplasmin level. Healthy volunteers with normal biochemical parameters served as controls [Ferritin 20.1 ng/ml (3.2–165.2); Tf saturation 26.51 ± 10.35%]. DNA was extracted from peripheral blood leucocytes. The coding regions and splice junctions of HFE, HJV, TFR2, HAMP and SLC40A1 genes were amplified and mutation screening was carried out using conformation sensitive gel electrophoresis (CSGE).Amplicons which showed a heteroduplex in CSGE was sequenced in an ABI 3130 genetic analyser to confirm the genetic changes. The median age of the patients was 52 (19–71). The changes observed in various genes involved in iron metabolism screened in this study are tabulated (Table 1). Apart from the common H63D HFE mutation seen in heterozygous state in two patients, a number of polymorphic sites ($N = 11$) were observed in different genes where 4 are novel. Low penetrance polymorphisms have been implicated in the clinical expression of complex diseases. In our study there was a significant difference in the distribution of $(CGG)_7$ and $(CGG)_8$ alleles between the patients and controls [$(CGG)_7$ 0.75 vs. 0.409; $(CGG)_8$ 0.25 vs. 0.59 ($P = 0.03$)]. $(CGG)_n$ repeats in 5′UTR play an important role in regulating the translational efficiency of the gene. $(CGG)_7$ repeat in SLC40A1 gene has been associated with haemochromatosis of African origin. The CA insertion in IVS 17 of TFR2 was shown to affect splicing by in silico methods. However, this insertion was also seen in the normal controls with a frequency of 0.3. Functional studies need to be performed to elucidate the role of these polymorphisms, which potentially contribute to iron overload. This is the first report from India on comprehensive screening of iron regulating genes in iron overload. The absence of mutations in these genes in Indian patients reveals that iron overload is linked to unidentified novel genetic factors.

Table 1 Genetic changes observed in patients with unexplained iron overload

GENE	HFE	HAMP	TFR2	SLC40A1
			EX15c.1851C>T−2 (Hetero)	
		IVS2(+12)	I238M−1 (Hetero)	$(CGG)_{7/7}$−4
		A>C[a]−1	EX18(−45)C>T[a]−1 (Hetero)	$(CGG)_{7/8}$− 4
GENETIC	H63D−2[b] (Hetero)		IVS 1(18)T>C−3 (Homo)	IVS1(−24)G>C−5 (Homo)
CHANGES (N)			IVS 17 ins CA[a]	EX6c.148T>C−1 (Hetero)
			Hetero-4	IVS4(+20)C>T[a]−2 (Hetero)
			Homo-1	

[a] Novel
[b] Mutation

Abstract 057

Langerhans' Cell Histiocytosis (with Multisystem Affection, Letterer Siwe Disease)

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Introduction Letterer-Siwe disease (a type of Langerhans' Cell histiocytosis) is a clinical syndrome characterized by systemic clonal proliferation of Langerhans' cells. It occurs mainly in children. Bone, skin and lymph nodes are the predilection sites. Patients with multisystem disease usually die from the disease. Gene expression profiling studies have shown high expression of MMP12 in multisystem Langerhans' cell histiocytosis and thus the gene may play a role in disease progression. Some older studies have suggested that the prognosis in Langerhans' cell histolysis can be predicted on the basis of histopathological features. Others have suggested that young age at diagnosis, hepatosplenomegaly, thrombocytopenia and polyostotic disease are associated with a poor prognosis. *Case Report* Here we present a case of Letterer siwe disease in an 11-month-old male child who presented with fever 5–6 days and deep yellow urine for a day. Patient developed conjugated hyperbilirubinemia and bilateral cervical and axillary lymphadenopathy. Conspicuous pallor and icterus marked. Skin revealed profuse petechiae and extensive seborrhoeic dermatitis. Abdomen was distended. Umbilicus everted and hepatosplenomegaly comfortably felt. Gingivitis and oral thrush also seen. Patient was alert, oriented but irritable. Imaging modalities exhibited no lesions in skull and chest X-rays. However USG showed hepatosplenomegaly and parenchymal echogenicity of kidneys. Histopathology of cervical lymph node showed complete replacement of nodal parenchyma by proliferating histiocytic cells in sheets. Vague sinusoidal pattern was seen at places. Nuclei of the neoplastic cells typically had longitudinal groove and were irregular contoured with thin nuclear membrane, delicate chromatin and inconspicuous nucleoli. Cytoplasm was abundant and highly eosinophilic. Eosinophils was scanty. No atypia seen. Mitotic index low. Immunohistochemistry of neoplastic cells revealed CD45 −Ve, CD 3 −Ve, CD 20 −Ve and S-100 +Ve. The diagnosis of Langerhans' cell histiocytosis with multisystem involvement was made (Letterer siwe disease). Patient died 3 months after diagnosis. *Discussion* Langerhans' cell histiocytosis present along a continuum of illness, ranging from indolent to explosive forms. Patient with multisystem disease experience organ failure, which is fatal. Several staging and scoring systems have been proposed. Most cite age ($<$2 years), extent of disease and the function of involved organs as the important prognostic variables.

Keywords Langerhans' cell histiocytosis, Letterer Siwe disease, Lymphadenopathy

Abstract 058

Molecular Basis of Bernard Soulier Syndrome in 26 Unrelated Patients in India: Identification of Two Common Ancestral Mutations and Their Genotype–Phenotype Correlations

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Bernard Soulier syndrome (BSS) is an extremely rare (1:1,000,000) hereditary bleeding disorder of platelet adhesion, caused by a qualitative or quantitative defect of glycoprotein (GP)Ib/IX/V complex. We report here the molecular basis of BSS in twenty-six unrelated patients from India. The diagnosis was based on low platelet count, presence of giant platelets and aggregometry studies (absence of response to ristocetin) in these patients who presented with mucocutaneous bleeding at 8.5 years of age (range 9 months to 60 years). Flow cytometry to assess surface GPIb/IX/V complex using anti-CD42 antibodies showed reduced (7.7–57%, $n = 12$) expression. Genomic DNA was screened for mutations in the GPIb alpha, GPIb beta, GP9 genes by PCR and conformation sensitive gel electrophoresis strategy. Mutations were identified in all the 26 patients. Fifteen different disease causing mutations, including missense (60%), frame shifts (33%) and nonsense (7%) mutations were identified of which 12 are novel. The molecular pathology of the five novel frame-shifts predicted by either insertions or deletions (GPIb alpha: c.127 InsGA, c.402-403InsC, GPIb beta: c.145_167del22bp, C.439_C.440 Dup38; GP9: c.86-87delG) or the one nonsense mutation (GP9, p.295, Glu>X) is evident as they predict premature termination of translation of the respective gene products. Among the five novel missense mutations, 3 were identified in a single patient (CD42b:21.1%). These included a novel p.492, Tyr>His in GPIb alpha gene in heterozygosity with p.129, Glu>His, p.132, Leu>Pro mutations in GPIb beta detected in a double homozygous condition. All these substitutions occur at highly conserved codons in the transmembrane region of GPIb alpha and beta chains, a region critical for anchoring the glycoprotein subunits into the platelet membrane. Another patient had a p.65, Pro>Arg missense substitution, which modified a conserved residue in the COOH-terminal region flanking the single-copy leucine-rich domain of GPIb beta. This substitution in the extracellular domain of GPIb beta showed reduced levels of not only the GPIb (10%) protein but also GPIX (25%) in the patient, suggesting it affects both the maturation of GPIb alpha as well as GPIX stability. A p.55, Phe>Cys missense change in homozygous state identified in two patient is conserved across GPIX of different species (murine, swine and macaques) and located within the leucine rich motif of GP9 gene. An alteration at this site is likely to lead to impaired surface expression of GPIb/IX/V complex. Interestingly, two common mutations affecting a total of 15 unrelated patients were identified in the present study. A novel deletion of 22 bp in exon 2 of GPIb beta (c.145_167del22bp) gene was responsible for the disease in nine patients, and a previously reported GP9, p.8, Cys>Arg missense change in six different patients. Haplotype analysis suggested a possible ancestral origin for these mutations and remained localized to Southern India. The molecular data presented here is the largest series of BSS patients reported from the world so far, adding significantly to the mutation database of this condition and also useful for its genetic diagnosis in India.

Abstract 059

An Interesting Hemoglobinopathy in a Bengali Family

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HbE is prevalent in the eastern part of India. We are presenting a case of a 35-year-old Bengali female who presented to us with a hemoglobin of 10.3 g/dl and microcytic hypochromic indices. Her HbHPLC revealed a predominance of HbA with HbA2/E window of ∼15.5 with a high HbF ∼20%. The alkali electrophoresis showed a band in A2/E region. On studying the family, her parents were found to have normal RBC indices. Her father's HbHPLC showed similar

peaks as the proband while the mother was normal. The son of the proband also showed similar peaks on Hb HPLC but the daughter was normal. So we had an interesting situation where three generations of a family showed similar findings. The amount of HbE was lower than expected for a heterozygous state and the HbF was high with a lack of symptoms. Here we will present the possible explanations for this interesting case.

Abstract 060

Diagnosis of Mastocytosis in a Case of AML

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Systemic mastocytosis is an uncommon entity but can occur coexistently with clonal hematopoietic disorders. This is a case of a 25-year-old male with AML-M2 associated with t(8;21) who was treated with 3 + 7 induction followed by three cycles of high dose cytosine arabinoside (HIDAC). His bone marrow post 2nd HIDAC, showed a hypocellular marrow with dense perivascular aggregates of elongated cells with clear cytoplasm. On toluidine blue staining, these aggregates showed metachromatic staining. We also examined his previous bone marrow biopsies including the diagnostic marrow. There were only a few perivascular aggregates of mast cells in all the biopsies examined. Hence a diagnosis of Systemic mastocytosis-associated hematopoietic non mast cell disorder was made. This is a new entity and the diagnosis is often missed at presentation due to the hypercellularity of AML. To conclude, SM-AHNMD is a newer entity and should be kept in mind with use of reliable tests for diagnosis.

Abstract 061

Co-Existing JAK2 V617F and BCR-ABL in a Case of Myeloproliferative Neoplasm

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A 34-year-old male presented with complaints of fever, weakness, decreased appetite, weight loss and feeling of fullness in the left hypochondrium for 6 months. On examination, he had hepatosplenomegaly with liver 4 cm below costal margin and spleen 12 cm below costal margin. On investigation he was found to have bicytopenia with hemoglobin of 5.7 g/dl and platelet count of $100 \times 10^3/$µl. His total WBC count was $10.46 \times 10^3/$µl. The peripheral smear showed a leukoerythroblastic blood picture with a shift to left in the neutrophilic series along with an occasional blast. The bone marrow aspirate was diluted with peripheral blood bone marrow biopsy showed grade IV myelofibrosis with large atypical clustered megakaryocytes. His peripheral blood sample was positive for both BCR-ABL and JAK2 V617F mutations. It is a very rare coincidence with only 12 cases reported so far.

Abstract 062

AML Associated with Tetraploidy and Extensive Erythrophagocytosis: A Case Report

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Numerical chromosomal changes have been described in AML patients, but hyperdiploidy, specially tetraploidy or near-tetraploidy, is very rare, also Erythrophagocytosis by neoplastic cells has been reported uncommonly. 18-year-old girl presented with severe menorrhagia to Hematology department, was diagnosed as AML M4 showed unusually large blasts with prominent erythrophagocytosis on BM examination and on karyotyping showed near tetraploidy. She died due to sepsis on D12 post induction. We report this case for its rarity.
Keywords AML, Tetraploidy, Erythrophagocytosis

Abstract 063

Low-Dose Thalidomide–Prednisolone in Myelofibrosis

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Background Allogeneic stem cell transplant is the only potentially curative treatment for Idiopathic myelofibrosis, but is often precluded by advanced age, poor performance status, co-morbidities and financial constraints. Low-dose thalidomide and prednisolone has been reported to be well-tolerated and effective in patients with myelofibrosis. We attempted to study the clinical outcome of patients treated with thalidomide–prednisolone at our centre. *Objective of the Study* To study the efficacy of low dose thalidomide with prednisolone in patients with idiopathic myelofibrosis treated between January 2005 and July 2010. *Methodology* The out patient records of patients with idiopathic myelofibrosis treated with thalidomide–prednisolone were retrospectively analysed. The patient and disease characteristics were recorded and response confirmed according to European Myelofibrosis Network (EUMNET) criteria. Thalidomide was administered at a dose of 50 mg daily for a period of 3–6 months in combination with oral prednisolone (0.5 mg/kg/day slowly tapered over 3 months). Jak2 V617F was screened using Allele Specific PCR method. *Results* Forty-five symptomatic patients (hemoglobin level <10 g/dl or symptomatic splenomegaly) with myelofibrosis were treated including 29 males and 16 females with a median age of 54 years (range 22–74 years). Two patients lost to follow up after initiating treatment with thalidomide and were excluded from analysis. The median follow-up from the time of diagnosis to initiation of therapy was 13 months (range 1–69). At diagnosis, 14 (31.1%) patients were transfusion dependent (mean red cell requirement: 9 units, range 1–48) while 9 (20%) had platelet counts $<100 \times 10^9/$l [median 148.5 (range 30–603)]. All patients except for 2 had palpable splenomegaly (median 8 cm; range 0–28). The *JAK2V617F* mutation was positive in 10 out of 21 patients (47.6%) tested. An objective response was seen in 32 (74.4%) patients according to EUMNET criteria which included complete response in 2 (4.6%), major response in 22 (51.1%), moderate response in 6 (13.9%) and minor response in 2 (4.6%) patients. Eleven patients (25.5%) showed no evidence of response. Among 14 patients who were transfusion dependent, 7 (50%) improved and became transfusion independent, six remained transfusion dependent and one lost to follow up. Among nine patients with thrombocytopenia (platelet count$<100 \times 10^9/$l), 6 (66.6%) experienced $\geq$50% increase in their platelet count. In 18 of 45 patients (40%), spleen size decreased by more than 50%. The median time to early response was 4 weeks (range 4–52 weeks) while the median time to peak response was 12 weeks (range 4–52 weeks).

At a median follow up of 13 months (range 1–69), 24 (55.8%) patients continued to maintain their response at the time of last followup and the rest had progressive disease. There was no significant difference in the response to therapy between *JAK2V617F* mutation positive and negative cases. *Conclusion* Low dose Thalidomide combined with prednisolone results in sustained improvement in anemia and splenomegaly in patients with myelofibrosis.

Abstract 064

Late Onset Neonatal Anemia due to Maternal Anti Kp[b] Induced Haemolytic Disease of the Newborn

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Background Anemia in a neonate is a diagnostic challenge for clinicians. If features suggestive of haemolytic disease of the newborn (HDN) (i.e. jaundice) are absent during perinatal period and reticulocytopenia is present, immune HDN is considered a remote possibility. We report here a case of HDN due to anti-Kp[b], manifesting as severe anemia at the age of 1 month with a symptom free neonatal period. Allo anti Kp[b] is a clinically significant antibody against high frequency red cell antigen Kp[b] of Kell blood group system. It is a rare antibody and only few cases of HDN of varying severity due to anti Kp[b] have been reported. *Aim* To diagnose and successfully manage a case of hemolytic disease of newborn due to anti Kpb with transfusion of anti Kpb positive blood. *Methodology* ABO and Rh D Blood grouping were done using standard methods by tube technique and monoclonal grouping reagents (Diamed, Switzerland). Direct antiglobulin test (DAT), irregular antibody screening and identification was done by polyspecific LISS Coombs Gel card (Diamed AG, Switzerland). Three cell screening panel (Diacell, Diamed AG, Switzerland) and 11 cell identification panel (ID Dia-Panel, Diamed AG, Switzerland) was used for identification. Extended phenotyping for minor red cell antigens, preparation of Di Thio Threitol (DTT) treated red cells (Kp[b] negative), monospecific DAT, acid elution (Gamma Elukit, Immucor Gamma, USA reagent) and titration studies were done using routine methods. *Results* The infant had presented at the age of 1 month with chief complaint of respiratory distress with increasing pallor, refusal to feed and irritability. There was hepatosplenomegaly but no icterus or lymphadenopathy. Normocytic normochromic anemia with reticulocytopenia was confirmed. A bone marrow aspirate showed normoblastic, mild dyserythropoietic erythroid hyperplasia with relatively reduced myeloid cells with normal maturation. Blood group of the infant was B, Rh D positive, DAT was 2+. Mother's blood group was A$_1$B, Rh D positive. Alloantibody to a high frequency antigen was detected in mother's serum and a screening and identification result was consistent with presence of antibody to high frequency red cell antigen. Anti Kp[b] was confirmed by using DTT treated red cells (Kp[b] negative) for antibody screening which gave negative reaction. Titre of the antibody was 8 at saline phase and 64 by IAT. Infant and father's red cell were kp (a−b+). In due course infant was transfused six times group B Rh(D), kp[b] positive PRBCs. Response to transfusions before under steroid cover was good. At 4 months of age the child was symptom free and did not require transfusion any more. DAT became negative at 6 months of age. *Conclusion* In conclusion, anti-Kp[b] appears to be capable of causing severe late HDN. Infants presenting with immune haemolytic anemia in first 6 months of life should be evaluated for a probable HDN even if the history is not suggestive. Also, infant born to irregular antibody positive mothers should be investigated for immune HDN in a neonate even if an infant is asymptomatic at birth and closely monitored for several weeks after birth. All pregnant women regardless of Rh D type should be screened for clinically significant red cell antibodies and managed accordingly.

Abstract 065

A Novel *β* Globin Gene Mutation in Codon 7 Produces a Hemoglobin Variant Mimicking HbS in HPLC

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Hemoglobin S (HbS) and *β* thalassaemia are highly prevalent haemoglobin disorders in the India. Laboratory diagnosis of these diseases is based on peripheral blood analysis and hemoglobin electrophoresis or cation exchange-high performance liquid chromatography that detects and quantitates different types of haemoglobins present in these patients. DNA analysis to detect molecular lesions present in the globin genes is required in where there is discordance of these results with the clinical phenotype. In this report, we describe a case of hypochromic microcytic anemia that had a novel hemoglobin variant that elutes in the S window in HPLC that could cause misdiagnosis without DNA analysis. The patient is a 6-year-old boy from West Bengal, referred to our department for the clinical evaluation for hepatosplenomegaly (liver 2 cm and spleen 1 cm). Hematological parameters were measured by an automated cell counter (LH 750, Beckman Coulter, USA). HbA, HbF and HbA2/E levels were obtained by CE-HPLC (VARIANT Bio-Rad, USA). DNA was extracted by standard protocol. Common *β*-thalassaemia mutations [Codon8/9(+G), Codon15 (G→A), IVS I-1(G→T), Codon30 (G→C), IVS I-5(G→C), IVS I-1(G→A), Codon41/42(-TCTT), Codon26 (G→A) (*β*^{E}) and Codon6 (A→T) (*β*^{S})] were screened by reverse dot blot (RDB). Common alpha (α) globin deletions [−α$^{3.7}$, −α$^{4.2}$, −^{SEA}, −^{MED}, −^{SA}] were screened by multiplex PCR followed by agarose gel electrophoresis. DNA sequencing was performed using cycle sequencing kit on ABI 3130 Genetic Analyzer. The patient had a hemoglobin level of 11.4 g/dl with a MCV 55.6 fl. The sickling test showed that it is negative in both patient and mother. Hemoglobin electrophoresis in the patient and the mother detected an abnormal band at the position of HbS in both. Mother had additional HbA2 and HbA bands and patient had only HbA2. HPLC analysis confirmed the presence of a haemoglobin variant eluted in the S window of the chromatogram and this abnormal haemoglobin was in the levels of 82.4% in the patient and 37.9% in the mother, respectively. The HbF levels were slightly elevated in the patient (2.2%) while it was normal in the mother (0.8%). DNA analysis by reverse dot blot identified IVSI-5(G→C) in the patient while *β*^{S} (Codon6 A→T) was not detected. DNA sequencing analysis revealed a novel missense mutation **G**AG→**C**AG in the codon 7 position of the beta globin gene present in compound heterozygous with IVS I-5(G–C). This mutation causes a replacement of glutamate by glutamine which results in the formation of a haemoglobin variant. Twenty-eight such hemoglobin variants that elute at the position of HbS in HPLC have been described. This report describes a novel mutation in the beta globin gene which produces a haemoglobin variant that co-migrates or co-elutes with HbS in electrophoresis or HPLC. DNA analysis in this family helped us to avoid a misdiagnosis and the study illustrates a probable diagnostic pitfall using HPLC system for the diagnosis of sickle cell disease in Indian population.

Abstract 066

A Novel 26 bp Deletion in Exon-1 of the β-Globin Gene Causing Thalassaemia Major

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β-Thalassaemia is an inherited genetic disorder characterized by reduced or complete absence of β-globin gene expression. This disease is highly prevalent in India with a carrier frequency ranging from 3.5 to 15% in general population. Molecular characterization of β-thalassaemia is essential for the prevention of the disease and for understanding the disease biology. In this report we describe a novel 26 bp deletion from Codon 6 to Codon 14 in the β-globin in a consanguineous family from Tamil Nadu. The patient was a 2-year-old boy evaluated for anemia and hepatosplenomegaly. He was transfusion dependent with 9 units of blood received from the age of 10 months till date. Hematological parameters were measured by an automated cell counter (LH 750, Beckman Coulter, USA). HbA, HbF and HbA2/E level were obtained by high performance liquid chromatography (VARIANT Bio-Rad, USA). These results in the parents are shown in the Table 1. DNA extracted by standard protocol was analyzed for common β-thalassaemia mutations [Codon 8/9(+G), Codon 15(G→A), IVS-I-1(G→T), Codon 30 (G→C), IVS-I-5(G→C), IVS-I-1(G→A), Codon 41/42 (−TCTT) and Codon 26(G→A) (βE)] were screened by reverse dot blot (RDB). DNA sequencing of beta globin gene was performed using cycle sequencing kit on ABI 3130 Genetic Analyzer. Co-inheritance of alpha thalassaemia that modulates the severity of the disease was tested by a multiplex PCR method that detects seven common deletions prevalent in our population. RDB results showed the absence of normal sequence in the Codon 8/9 region. DNA sequencing of the β-globin gene revealed a 26 bp deletion (AGGAGAAGTCTGCCGTTAC TGCCCTG) from Codon 6 to Codon 14 in the homozygous state. Analysis in both the parents showed that they were heterozygous for the same mutation. This novel mutation causes a shift in the normal reading frame of the β-globin coding sequence and creates a stop codon at the position of codon 21. Premature termination of translation causes β0 thalassaemia in this patient. Deletion mutations are relatively uncommon in β-thalassaemia and they have to be documented as such molecular lesions can affect the molecular diagnostic approaches that mainly focus at detection of point mutations. Our study illustrates that there is a great diversity in the spectrum of mutations in Indian population and comprehensive screening and identification of new and rare alleles is important for genetic counseling and prenatal diagnostic programs in the country.

Table 1 Hematological parameters of parents of the patient homozygous for 26 bp deletion in the beta globin gene

	Hb (g/dl)	MCV (fl)	Reticulocyte count (%)	HbF (%)	HbA2 (%)
Father	11.1	68.9	1.860	6	
Mother	9.7	662.63	2.5	5.3	

Abstract 067

Macrothrombocytopenia in North India: An Underdiagnosed Entity

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Background Macrothrombocytopenia is an underdiagnosed entity and is seen in a group of inherited disorders characterised by low to normal platelet counts. Although this entity has been reported among the patients in eastern India, there is no report from North India. This study highlights the presence of this entity in North India and the role of mean platelet volume (MPV) and platelet cytogram pattern in the diagnosis of macrothrombocytopenia. *Aim of the Study* To determine the role of MPV and platelet cytogram pattern in the diagnosis of macrothrombocytopenia and to study the geographical distribution of these patients. *Case Series* Fourteen patients whose blood counts were run on Advia-120, a five-part automated hematology analyser were detected to have macrothrombocytopenia. The age of the patients ranged from 25 to 56 years. Eleven of the 14 patients were males. All patients were detected to have incidental thrombocytopenia. None of the patients had a past or family history of bleeding. Except for one patient, all other patients had normal hemoglobin, red cell indices and white cell count. The platelet count ranged from 59×10^9/l to 141×10^9/l (mean $86.6 \pm 24.0 \times 10^9$/l). Peripheral smear in all patients showed thrombocytopenia and large platelets. The mean platelet volume (MPV) ranged from 15.1 to 23.3 fl (mean 19.1 ± 2.2 fl) [normal 7.0–11.0 fl]. Data plots from all patients showed characteristic high volume platelet cytogram pattern with uniform distribution of large platelets and platelet flagging for large platelets (+++). The mean MPV was significantly higher ($P < 0.05$) than that seen in 29 patients with ITP (mean MPV 10.8 ± 3.5 fl) and in randomly selected 56 patients with secondary thrombocytopenia (mean MPV 10.9 ± 2.6 fl). The geographical distribution of these patients showed that 11 (78.5%) were from North India and 3 (21.5%) were from eastern India. *Conclusion* Macrothrombocytopenia is an under reported entity especially among the North-western population in India. Knowledge of this entity and optimal use of automated haematology data can aid in its diagnosis to avoid inappropriate investigations and treatment.

Abstract 068

Hepatosplenic Gamma/Delta Lymphoma: An Entity Which can Mislead the Clinician. A Case Report

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Introduction Hepatosplenic T-cell lymphoma (HSTCL) is a rare peripheral T-cell lymphoma characterized by occurrence in young adult males with hepatosplenomegaly, B-symptoms, peripheral blood cytopenias, absence of lymphadenopathy; lymphomatous infiltrates in the splenic red pulp, hepatic sinusoids, and bone marrow sinuses; T-cell receptor (TCR) γδ chains and an aggressive clinical course. Bone marrow involvement although common, may be subtle in bone marrow specimens. In view of the non-specific clinical features, this entity may be missed on routine blood and bone marrow evaluation and requires a high index of clinical suspicion for correct diagnosis. *Case Presentation* We report a 22-year-old gentleman who presented with gum bleeding and found to have anemia, thrombocytopenia and massive hepato-splenomegaly. The bone marrow evaluation was suggestive of peripheral destruction of platelets and he commenced on steroids with transient improvement. He presented 2 months later

with intermittent fever, malaise, hepatosplenomegaly, anemia and thrombocytopenia. Although evaluation for disseminated TB and malaria was negative, he was initiated on empirical Anti-tubercular treatment. He presented 3 months later with persistent fever. In view of a high clinical suspicion of HSTCL, a diagnostic splenectomy was performed and immunohistochemistry was conclusive of HSTCL. *Conclusion* HSTCL can prove to have a confusing clinical presentation and requires a high index of suspicion.

Keywords Hepatosplenic gamma/delta lymphoma, High clinical suspicion, Hepatosplenomegaly, Splenectomy

Abstract 069

Awareness/Understanding on Haematopoietic Stem Cell Transplantation Among Doctors from Tertiary Care Hospitals in North India

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Background Haematopoietic stem cell transplantation (HSCT) can be curative for many haematological diseases. While more than 300 HSCTs are done every 10 million population in developed countries, the number in India is only <3 every 10 million population. Although economics play a role in this difference, another reason is the lack of awareness/understanding of the doctors and appropriate referral to a transplant centre. We undertook this study to assess the awareness/ understanding of HSCT among doctors working in three tertiary care hospitals in Punjab. *Aim of the Study* To determine the level of awareness/understanding among doctors about haematopoietic stem cell transplantation. *Methodology* A prospective cross sectional questionnaire based study was conducted among 185 doctors over a period of 2 months from three different medical colleges in Punjab. The level of awareness/understanding was assessed on the basis of differential scoring done on 25 questions. *Results* Nearly all (99.4%) of doctors were aware of HSCT. However, the categorization of level of awareness/understanding showed that 5% of doctors had good understanding, 20% had adequate understanding, 59% had partial awareness and 16% had poor awareness. According to the years of experience after internship, good/adequate understanding was only found among 21% of doctors with >30 years experience, 20% of doctors with 20–29 years of experience, 45% of doctors with 15–19 years of experience, 29% of doctors with 10–14 years of experience and 26% of doctors with 1–9 years of experience. *Conclusion* Although most doctors are aware of HSCT, the level of awareness/understanding requires improvement for timely referral to transplant centers.

Abstract 070

Intramedullary Cryptococcoma in Acute Lymphoblastic Leukemia Presenting as Brown-Sequard Syndrome: A Case Report

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Introduction Cryptococcus neoformans (*C. neoformans*) is a thin-walled, non-mycelial, budding yeast that is ubiquitous and causes opportunistic and non-contagious infections in humans primarily through airway inhalation. Involvement of the central nervous system (CNS) has been found in 70% of patients at the time of diagnosis, and meningitis and meningoencephalitis are the most common manifestations. Space-occupying cryptococcoma, is a rare entity, characterized by localized, solid, tumor-like masses that are usually found in the cerebral hemispheres or cerebellum, but are extremely rare in the spinal cord. *Case Summary* We report a 19-year-old boy with acute lymphoblastic leukemia who presented with a right sided hemiparesis associated with a contralateral sensory deficit, consistent with a Brown-Sequard syndrome. MRI of the cervical spine was consistent with an intramedullary rounded space occupying lesion at the level of C2 right hemicord. Diagnosis was made by a positive India Ink CSF study and a positive latex agglutination test for cryptococcal antigen. *Conclusion* Intramedullary cryptococcoma is a rare occurrence and its presentation as Brown-Sequard syndrome is even rarer. In patients with leukemia a CNS lesion is almost always considered to be a leukemic deposit and screening for cryptococcosis is important.

Keywords Intramedullary cryptococcoma, Cryptococcosis, Magnetic resonance imaging

Abstract 071

Prevalence of Arthropathy Among Patients with Haemophilia in a Tertiary Care Comprehensive Haemophilia Centre

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Background Managing hemophilia today needs a multidisciplinary approach. Physical therapy is an indispensable and important component of care for such patients. However this mode of therapy is used sparingly in most patients leading to significant disabling joint problems. *Aim of the Study* To determine the prevalence of arthropathy in patients with haemophilia registered with a comprehensive haemophilia care centre in a tertiary care hospital. *Methodology* A survey was done in a tertiary care comprehensive haemophilia centre in North India. Forty-six patients with hemophilia registered for the survey. Patient information on history, clinical profile and treatment received were recorded. Blood sampling for factor assays was done along with a detailed joint assessment for each patient. *Result* Of the 47 patients, 40 (85.1%) had Hemophilia A and 7 (14.9%) patients had Hemophilia B. Among patients with Hemophilia A, 7 had mild disease, 18 had moderate disease while there were 22 patients with severe hemophilia based on factor VIII levels. Among 7 patients with Hemophilia B, one had mild haemophilia, two had moderate hemophilia and four patients had severe hemophilia with factor IX levels <1%. Out of 40 patients with Hemophilia A, 23 (57.5%) patients had joint problems while 6 (85.7%) out of seven patients with Hemophilia B had joint problems. The most common problems were arthrosis of the knee, fixed flexion deformity of the elbow and knee. *Conclusions* Comprehensive haemophilia care through multidisciplinary approach is vital to reduce joint related morbidity of haemophilia patients. This study highlights the prevalence of the joint problems so that definitive steps can be taken to reduce the morbidity.

Abstract 072

Post Splenectomy Lymphocytosis: A Case Report

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Introduction Post splenectomy lymphocytosis is an extremely rare entity. However, it is important to recognize this entity especially when certain other clinical conditions are associated with it where the absolute neutrophil count and lymphocyte counts influence clinical decision making. *Case report* A 25-year-old lady presented with nausea and vomiting. Routine evaluation showed hepatitis C infection (Genotype 1—copy numbers: 5.99×10^6IU/ml). She had a splenectomy done at the age of 12 years for thalassaemia intermedia syndrome when she had received 15 transfusions. Further laboratory evaluation revealed total leucocyte counts ranging from 8,000 to 10,000/cu mm with lymphocyte percentage of 60–70% and absolute lymphocyte counts varying between 4,000 and 7,000/cu mm over a period of 4 months. Peripheral blood Immunophenotyping revealed a T cell population with no clonal abnormality. Bone marrow examination showed erythroid hyperplasia with no lymphocytosis in the bone marrow. The patient was subsequently started on Ribavirin and interferon alpha for treatment of Hepatitis C infection. *Conclusion* Hepatitis C can be associated with lymphoproliferative disorders and it is essential to rule out clonal abnormalities in such a patient with lymphocytosis. The knowledge of post splenectomy lymphocytosis is essential to make the right management decisions. Whether spleen plays a role in the absolute lymphocyte number needs to be evaluated further.

Abstract 073

Cerebrovascular Diseases (CVD) and Coronary Artery Syndromes (CAD): Should they be Screened for JAK2(V617F) Mutation?

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Background Natural history of myeloproliferative neoplasia (MPN) reveals that 64% of thrombotic events occur either at presentation or before diagnosis. Recognition of the somatic mutation JAK2 (V617F) in 2005 has unified all myeloproliferative disorders. This gain-of-function mutation is especially seen in polycythemia vera (PV), essential thrombocytosis (ET) and idiopathic myelofibrosis (IMF). *Case series* We present three patients who presented with thrombotic events and later noted have JAK2 (V617F) mutation positive ET and PV. The first patient presented with coronary artery disease at the age of 39 and 14 years later presented with re-occlusion of the coronary stent. Subsequent evaluation confirmed essential thrombocytosis. The second patient was a 28-year-old lady who presented with history of transient ischaemic attack 2 years prior to presentation and recurrent abortions. On evaluation she was found to have JAK2 (V617F) mutation positive PV. The third patient was a 51-year-old man who presented with gangrene of right little and ring fingers 1½ years prior

to presentation of right middle cerebral artery infraction. He was later found to have JAK2 (V617F) mutation positive PV which was masked by iron deficiency. Two of the three patients had splenomegaly at the time of diagnosis with no obvious secondary causes of polycythemia and thrombocytosis. *Conclusion* JAK2 (V617F) mutation screening may be considered to be included in the work up of thrombotic events especially in the young. With targeted therapy with small molecules against this mutation is round the corner, patients with this mutation may benefit. **Keywords** Myeloproliferative neoplasia, Polycythemia vera, Essential thrombocytosis, JAK2 (V617F) mutation

Abstract 074

Standard Busulphan/Cyclophosphamide/ATG Regimen as an Option for Conditioning in for a One Antigen Mismatch Allogeneic Stem Cell Transplant with Father as Donor in a Patient with Wiskott Aldrich Syndrome (WAS): A Case Report

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Introduction Wiskott-Aldrich Syndrome (WAS) is an X-linked recessive disorder characterized by a triad of thrombocytopenia with decreased mean platelet volume, eczema, and increased susceptibility to pyogenic and opportunistic infections. Hematopoietic stem cell or cord blood transplantation is the only curative therapy for WAS. Recipients of transplants from HLA-matched sibling donors have a 5-year survival rate of approximately 90 percent. In the absence of a sibling and when the option of matched unrelated donor transplant is non-available, a HLA mismatched transplant can be considered. *Case presentation* 1½ year old boy presented with thrombocytopenia, recurrent episodes of diarrhea and eczema since birth. He was diagnosed to have Wiskott Aldrich Syndrome with mutation of 343-344 del (−C) on Exon 10 of X chromosome. (single nucleotide deletion, [c, 1061-1065 del C, Pro343-344 fsX 444], A novel nucleotide variation resulting in truncated protein). We report a one antigen mismatch allogeneic stem cell transplant performed with father as the donor in a patient with WAS. Modified busulfan/cyclophosphamide/ATG conditioning was used with ATG scheduling done along with cyclophosphamide for a probable in vivo T cell depletion of the donor T cells. The patient engrafted on day +25. However, he had a grade III skin GVHD which responded to Daclizumab, Mycophenolate and continuation of cyclosporine and steroids. Fifteen months following transplant, he is off steroids and cyclosporine with tapering doses of mycophenolate with no evidence of GVHD. *Conclusion* In the absence of HLA identical siblings, one antigen mismatch parent can be a stem cell donor in patients with Wiskott Aldrich syndrome.

Abstract 075

Blood Culture and Antimicrobial Susceptibility Pattern of Patients in Haemato-Oncology Unit

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Aim of the Study To determine the blood culture and antimicrobial susceptibility pattern of patients admitted under the haemato-oncology unit in a tertiary care hospital. *Methodology* Blood culture and antimicrobial susceptibility pattern of all the patients from clinical haematology unit were analyzed retrospectively over a period of 12 months. *Results* A total of 345 blood cultures were sent for 155 patients. Out of this 47 (13.6%) blood cultures were positive among 38 patients and the spectrum of organisms were; 25% *Staphylococcus aureus* (15% Methicillin Resistant *Staphylococcus aureus*, 10% Methicillin Susceptible *Staphylococcus aureus*) 7% Coagulase negative staphylococcus, 21% *E. coli*, 15% Enterobacter species, 10% Klebsiella species, 10% Pseudomonas species, 4% Enterococcus species, 2% Acinetobacter species, 2% *Streptococcus viridans* and 4% *Candida albicans*. The patient profile was as follows: haematological malignancies: 60%, aplastic anemia: 13%, stem cell transplant: 11%, non malignant haematological disorders: 16%. Antibiotic susceptibility patterns showed 10% carbapenem resistance in the E coli strains and 68% potential ESBL producers in the family Enterobacteriaceae. *Conclusion* It is important to understand the clinical profile, blood culture and susceptibility pattern of patients in every haemato-oncology unit. This will help the clinician to choose the appropriate treatment.

Abstract 076

Flowcytometric Enumeration of CD34+ Cells by a Modified ISHAGE Method

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Haematopoietic stem cell (HSC) quantification is critical for haematopoietic stem cell transplantation (HSCT). CD34 antigen is the best available marker of HSC and is widely used for enumeration of this population. At our center we evaluated a dual platform modified ISHAGE protocol, which is easy to establish from both a technical and personnel perspective, by comparison with data provided as part of an external quality control program that we participate in (conducted by the Royal College of Pathologists of Australia). Viable cells (R1) are displayed in CD45 vs. SSC dot plot, so as to contain all CD45$^+$ events including CD45dim and CD45bright (R2) and CD45$^-$ events were excluded(i.e. red blood cells, platelets and other debris). Cells forming a cluster with characteristic low SSC are then gated on this second plot to produce a third region (R3). The third plot which is CD45 vs. CD34 is obtained by plotting the events from gates R1, R2 and R3 which gives CD34% on gate R4 (summarized in Fig. 1). CD34 analysis was done by staining the whole blood/bone marrow/PBSC product samples with CD45 FITC and CD34 PE. This assay simultaneously enumerates the total viable dual positive (CD45$^+$/CD34$^+$) HSC in absolute counts of CD34$^+$ cells/µl and the percentage of viable leucocytes(CD45$^+$) that are CD34 positive. The total CD34+ cell number in the cell suspension available for transplantation is determined by multiplying the fraction (percentage) of CD34+ cells by the total leukocyte number (obtained on a separate platform). CD34 percentages were also generated by using a conventional ISHAGE gating strategy for comparison. We compared 14 samples received from the external quality assessment program, between August, 2006 and July, 2010. The median value of CD34% generated at our centre by modified ISHAGE was 0.19 (range 0.05–0.64) and by ISHAGE method was 0.20 (range 0.05–0.7). The CD34% obtained by modified ISHAGE and the conventional ISHAGE gating strategy were compared with the data provided by the external quality control program and was found to be not significantly different ($P = 0.000$; Intra-class Correlation Coefficient = 0.098). In conclusion this simple dual platform modified ISHAGE method provides an easily reproducible method by which we can enumerate CD34$^+$ cells. This method although simple was extremely accurate as compared to the conventional ISHAGE protocol.
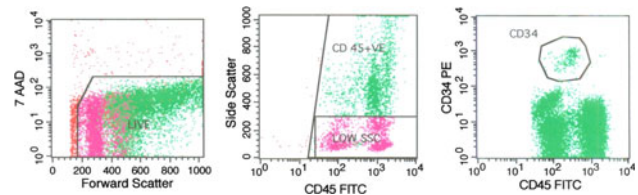

Fig. 1 Gating strategy for CD34 enumeration

Abstract 077

"Red Spots and Green Stains!"

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Introduction Idiopathic thrombocytopenic purpura (ITP) is a diagnosis of exclusion. Spleen is the site of peripheral destruction of platelets in ITP and is usually not enlarged. Splenectomy is reserved for cases unresponsive to conventional treatment. Amyloidosis is characterized by extracellular deposition of eosinophilic protein in various organs and can either be systemic or primary leading to its various manifestations. Systemic amyloidosis involves multiple sites and is usually secondary to long standing diseases. Bleeding manifestations in amyloidosis are usually due to coagulation factor deficiencies. Rare associations of bleeding in the absence of coagulopathy have been reported secondary to platelet dysfunction. There is however, no known association of amyloidosis with thrombocytopenia. Although there are few case reports on isolated amyloidosis of spleen, to our knowledge there is no other case report on ITP with amyloidosis of spleen. *Case Presentation* We report a patient who presented with epistaxis and purpura for 1½ months. Baseline evaluation showed thrombocytopenia and bone marrow confirmed the diagnosis of ITP. He underwent a splenectomy in view of inadequate response after treatment with IVIG, steroids, dapsone. Gross examination of spleen showed weight of 180 g and it measured 11 × 7 × 3.5 cm. Histopathology showed prominent amyloid deposits which were congophilic, birefringent and auto-fluorescent with thioflavin T obliterating the sinusoidal structures. Two years post-splenectomy, the platelets have maintained more than 2 lakhs and the patient is doing well. Investigations for systemic disease, light chain disorder, multiple myeloma and collagen vascular disease were negative. *Conclusion* Isolated splenic involvement in amyloidosis in itself is a rare entity, and there is no documented association of splenic amyloidosis and ITP. We hereby report this rare association of isolated splenic amyloidosis and idiopathic thrombocytopenic purpura.

Abstract 078

Dental Arch Dimensions in Beta Thalassaemia Major

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Background Patients with thalassaemia major can have major oral changes due to enlargement of the maxilla caused by bone marrow expansion. Affected patients usually suffer from spacing of the teeth and forward drift of the maxillary incisors. There have been only anecdotal reports of dimensional changes of the dental arch in patients with the disease. However, few studies have biometrically quantified these changes. *Aim of the Study* The aim of this study was to examine the arch dimensions of children and young adult patients with β-thalassaemia major in Ludhiana in comparison with an unaffected control group. *Methodology* The sample consisted of 10 patients with β-thalassaemia major (mean age = 11.5 ± 4.5 years) and an unaffected control group (mean age = 11.5 ± 4.5 years) matched for dental age, sex, and incisor and molar relationships. Measurements were made using a vernier calliper on stone models. *Results* In the maxilla, comparison of the arch depth, intercanine width and intermolar width showed narrower and shorter measurements among the patients. However, the difference was not statistically significant. (Arch depth $P = 0.258$, Intercanine width $P = 0.445$, Intermolar width $P = 0.188$). Measurements in the mandible showed smaller incisor widths among the patients with insignificant P value. (Arch depth $P = 0.857$, Intercanine width $P = 1.116$, Intermolar width $P = 0.971$, Interincisal width $P = 0.323$). *Conclusion* Patients exhibited a narrower and shorter maxilla; smaller incisor widths for the maxillary and mandibular arches. A study of larger number of patients may be required for documenting statistically significant variations.

Abstract 079

Atraumatic Rupture of Spleen Following Rituximab Therapy: A Case Report

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Introduction Spontaneous splenic rupture is described in patients with infections and occasionally with haematological malignancies. Rupture of spleen following treatment of lymphoma with rituximab is extremely rare. *Case Report* A 68-year-old gentleman was diagnosed with Diffuse Large B cell Lymphoma (DLBCL) and had achieved complete remission on PET scan following 6 cycles of R-CHOP chemotherapy. He presented 8 months later with abdominal discomfort and evaluation showed massive abdominal lymphadenopathy and splenomegaly suggestive of a relapse. In view of relapse he was planned for Rituximab-DHAP chemotherapy followed by autologous stem cell transplant. However, 1 h after initiation of 100 mg Rituximab on the first day, he developed severe abdominal pain, transient hypotension with tenderness in the left upper quadrant. Subsequent infusion of rituximab was withheld. Ultrasound showed significant free fluid in the abdomen, which on aspiration was found to be frank blood. An urgent laparotomy revealed haemoperitoneum and a rupture of the spleen near the upper pole. Histopathology of the spleen showed markedly friable tissue. Immunohistochemistry confirmed splenic involvement by diffuse large B cell lymphoma. *Conclusion* Rituximab is increasingly being used in B cell lymphoproliferative disorders. Knowledge of this rare adverse effect is important while using this drug. Atraumatic splenic rupture can be an easily overlooked complication of lymphoma especially during the first cycle of Rituximab when the tumor burden is large.

Abstract 080

Characterization of Leukemias Using WBC Flags and Scatterplots Generated by Advia-120 Automated Haematology Analyzer

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Background Modern day five part differential automated haematology analyzers can characterize the white cell population with greater detail than earlier. However, most of the valuable automated graphical and flagging data related to white cells is under-utilized. *Aim* To study the WBC scatterplots and morphology flagging data in acute and chronic leukemias in Advia-120. *Methodology* This was a prospective study carried out in the haematology laboratory of a tertiary care hospital. Blood samples received for complete blood counts from 66 patients with acute and chronic leukemia were run on Advia-120 automated analyzer. The instrument uses light scatter and myeloperoxidase staining for white cell differential count and, in addition, has a large unstained cell (LUC) zone for atypical cells/blasts. Peripheral smear examination, bone marrow studies and cytochemistry was done along with immunophenotyping and other ancillary tests wherever required. *Results* The study included twenty-nine patients with acute leukemia and 44 with chronic leukemia. Of the 29 patients with acute leukemia, 21 had acute myeloid leukemia (AML) and 7 had acute lymphoblastic leukemia. One patient had biphenotypic leukemia. There were no patients with AML M0, M6 and M7. Of the 44 patients with chronic leukemia, there were 14 patients with chronic lymphocytic leukemia (CLL), 7 with related disorders (CLL-PLL and PLL) and one each with lymphoma spill over and plasma cell leukemia. There were 16 patients with chronic myeloid leukemia (CML) of which five were in blast crisis. Scatterplots in all six patients with AML M2 showed a high side scatter pattern and higher neutrophil counts compared to those in all the seven patients with AML M1 due to the presence of more number of myeloperoxidase positive blasts. Both patients with acute promyelocytic leukemia showed near identical characteristic parabolic scatterplots with very high peroxidase activity. In all seven patients with monocytic/monoblastic leukemia, monocytic cell percentage was increased. No differentiation between scatterplots in six patients with AML M4 and a single one with AML M5 was seen. Of the seven patients with ALL, three with ALL-L1 had scatterplots showing high lymphocyte population in view of the small blast size although the blast and/or atypical flag were generated. All four patients with ALL-L2 showed high LUC count. All patients with chronic phase CML showed high side scatter pattern with low LUC count and consistent immature granulocyte flagging. Patients with blast crisis, in addition, showed rising LUC counts signifying increased blast cells. All 14 patients with CLL showed cell plots mainly in the lymphocyte zone on the scatterplot while those with CLL-PLL, PLL, lymphoma spill over and plasma cell leukemia, in addition, showed high LUC counts. *Conclusion* WBC scatterplots in combination with the flagging

information provide a novel way to evaluate and pre-empt diagnosis in leukemias. Although they cannot replace the eye count differential, they are useful in immediately suggesting further appropriate tests. **Keywords** Acute, Automated, Chronic, Leukemia, LUC, Scatter

Abstract 081

High Dose N Acetyl Cysteine (NAC) as a Treatment Option for Severe Sinusoidal Obstruction Syndrome (SOS) in a Patient with Thalassaemia Major Undergoing Allogeneic Stem Cell Transplant: A Case Report

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Introduction Hepatic Sinusoidal Obstruction Syndrome (SOS) is a clinical syndrome consisting of jaundice, ascites and/or unexplained weight gain, and hepatomegaly and/or upper-quadrant abdominal pain. This is especially seen with busulphan/cyclophosphamide conditioning in allogeneic stem cell transplant. Despite aggressive therapy, severe SOS is almost uniformly fatal. The most effective therapy is defibrotide which is not readily available in India. We report a case of hepatic SOS who responded to high dose N acetyl cysteine (NAC). *Case presentation* 14-year-old girl with thalassaemia major in Lucarelli class III underwent allogeneic stem cell transplant with her HLA identical brother as the stem cell donor. The conditioning used was Busulphan/cyclophosphamide/ATG. On day +8 she developed SOS which progressed over the next 3 days with a maximum bilirubin of 25 gm/dl (day +11). As Defibrotide was not readily available, we used high dose N Acetyl Cysteine similar to the dosing strategy of acetaminophen poisoning from day +10. There was immediate response with arrest in progression of bilirubin and further improvement in the next 2 days and bilirubin reduced to 18 gm/dl before initiation of defibrotide. *Conclusion* High dose NAC may be considered as a treatment option in severe SOS when there is non-availability of defibrotide.

Abstract 082

Heterozygous Beta Thalassaemia with Congenital Dyserythropoietic Anemia: A Case Report

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Introduction Congenital dyserythropoietic anemias (CDAs) comprise a group of rare hereditary disorders of erythropoiesis, characterized by ineffective erythropoiesis and distinct morphologic abnormalities in the erythroblasts on electron microscopy. Its interaction with beta thalassaemia trait has rarely been described. *Case Report* A 37-year-old gentleman presented with recurrent episodes of yellowish discolouration of sclera since childhood. Haemoglobin done on various occasions ranged from 8 to 10 gm%. There was no history of

blood transfusions in the past. Examination revealed pallor and icterus with moderate hepatosplenomegaly. Laboratory evaluation showed Hb: 9.6 gm%, MCV: 68 fl, RDW: 17.5% (CV) with other normal cell lines. Peripheral blood examination showed peripheral blood, moderate red cell anisopoikilocytosis with macrocytes, microcytes and basophilic stippling. Liver function test revealed marked direct hyperbilirubinemia. USG abdomen revealed chronic calculous cholecystitis and splenomegaly. HPLC was suggestive of beta thalassaemia trait. Upper GI endoscopy and Doppler ultrasound were normal. Bone marrow examination revealed hypercellular marrow with marked erythroid hyperplasia, with significant dysplasia: nuclear bridging, nuclear budding and severe delay in haemoglobinization. Electron microscopic examination showed widened nuclear pores, nuclear bridging, multi-lobed nuclei and Swiss cheese appearance and no peripheral duplication of endoplasmic reticulum. Possibility of CDA III was considered in view of negative Ham's test and multi-lobed nucleus. Cholecystectomy and splenectomy was done. He is on regular follow up and maintaining hemoglobin >9 gm%. *Conclusion* The possible interplay of two defective genes may have caused the unusual presentation. An entity called dyserythropoiesis/thalassemia syndrome can be considered in this patient.

Abstract 083

Eczematoid Palmoplantar Graft Versus Host Disease in a Sex Mismatched Allogeneic Stem Cell Transplant: A Case Report

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Introduction Chronic cutaneous graft versus host disease (cGVHD) can be classified as scleroderma-like and lichenoid forms. The initial presentation is sometimes subtle and a variety of less common cutaneous manifestations are reported. Palmoplantar cGVHD is a rare type of chronic cGVHD usually associated with eczematous dermatitis. *Case Report* A 25-year-old gentleman presented with bleeding gums, purpura and fever for 2 months and severe anemia requiring 10 blood transfusions. Baseline haematological evaluation and bone marrow examination confirmed aplastic anemia. He underwent allogeneic stem cell transplant as a curative option with HLA identical sister as the donor. Neutrophils engrafted on day +16. On day +115 he showed signs of dyshidrotic eczema and was initiated on local tacrolimus, steroids and systemic steroids. As there were features of cyclosporine induced MAHA, the same was stopped and oral mycophenolate was initiated on day +129. On Day +170 he presented with extensive progression of cutaneous and hepatic GVHD. Subsequent treatment with Cyclophosphamide, Sirolimus and Daclizumab did not show significant response. On day +195 he succumbed sepsis with multiorgan failure. The diagnosis of eczematoid GVHD was confirmed by cutaneous manifestions, biopsy, the clinical course and the presence of GVHD in other organs. *Conclusion* Eczematoid GVHD with palmoplantar lesions is associated with considerable morbidity and mortality. Early escalation of immunosuppression may be an option to achieve better control.

Abstract 084

Immunophenotypic Distinction Between Acute Myelogenous Leukemia with Thymic Markers [AML(T)] and T-Acute Lymphoblastic Leukemia with Myeloid Markers [T-ALL(M)]

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Background Blast in 20–40% cases of AML show T-lymphoid anti-
gens. Similarly, 20–30% of T-ALL cases express myeloid antigens.
This aberrant expression of lineage-associated markers by blast cells
poses diagnostic dilemma in the initial work up these cases. Therefore,
we wanted to identify additional immunophenotypic features that
could allow an accurate diagnosis in these cases by the commonly
applied primary antibody panel. *Materials and Methods* Seven hundred
twenty-eight cases of acute leukemia (AL) were immunophenotyped
using a comprehensive panel of 3- to 4-colour antibody combinations
directed against CD2, CD3, CD4, CD5, CD7, CD8, CD10, CD13,
CD14, CD15, CD19, CD20, CD22, CD33, CD34, CD41, CD45, CD61,
CD117 and HLA DR. Presence of cytoplasmic (c) CD3 and cMPO was
checked in some cases for confirmation of T-lymphoid and myeloid
phenotypes respectively. *Results* Sixty-one out of 323 cases of AML
(19%) and 18 out of 59 cases of T-ALL (30%) showed the presence of
aberrant T-lymphoid and myeloid markers respectively. Surface anti-
genic properties suggesting "myeloid" phenotype of blasts in AML(T)
were HLA-DR positivity (59/61; 97%), CD117 expression (57/61;
93%) and absence of CD10 (61/61; 100%). In contrast, "T-lymphoid"
phenotype in T-ALL(M) was indicated by normal intensity CD45
expression (100%), HLA-DR negativity (16/18; 89%), strong CD7
expression (17/18; 94%) and a high incidence of CD 13 negativity (11/
18; 61%). After applying these criteria, five cases of true mixed
myeloid/T-lymphoid leukemia (out of 79 cases with myeloid and T-
lymphoid markers) could be identified. *Conclusions* The above
approach could help in distinguishing cases of AML(T) from those of
T-ALL(M) at the level of primary immunophenotyping and could be a
forerunner for developing a scoring system for this purpose.

Abstract 085

HLA-B27 Typing: A Comparative Analysis of Common Flow Cytometric Methods

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Background Flow cytometric evaluation of HLA B27 is widely used
for the diagnosis of ankylosing spondylitis because of a strong
association between the two. A number of flow cytometric reagents
and methods are available for this purpose. However, cross-reactivity
of anti-HLA B27 antibodies with HLA B7 complicates interpretation
of results in most of these assays. PCR-based assays are considered
gold standard but are not easily available. Hence we compared two
commonly used commercial reagents for immunophenotypic detec-
tion of HLA B27 in leucocytes with a PCR-based assay to decide
about the preferred flow cytometric method. *Materials and Methods*
Twenty-seven samples were tested in parallel using a combination of
anti-HLA B27 (clone ABC-m3) and anti-HLA B7 antibodies tagged
with FITC and PE respectively [reagent A] and a kit containing a pre-
standardized calibrator, anti-CD3 antibody (FITC labelled) and an
anti-HLA B27 antibody (clone GS145.2) (PE labelled) [reagent B]. In
the latter method HLA B27 is assessed on CD3 positive lymphocytes
while in the former the total lymphocyte population is examined using
forward and side scatter plots. A PCR-based assay was performed in
all cases. *Results* Results with reagent A showed a 74% concordance

with those of PCR while this was 100% with reagent B. The high
degree of correlation between the results of reagent B with those of
PCR assay appears to be due to the high specificity of the anti-HLA
B27 (clone GS145.2) used in reagent B. Of the 12 cases with dual
expression of HLA B27/B7 and weak HLA B27 positivity by reagent
A, all 7 with low MFI were B27 negative by reagent B and by PCR.
This false positivity was due to cross-reactivity of antibody clone
ABC-m3 with the product of HLA B7 03 allele as evident from PCR
assays. *Conclusions* Use of anti-HLA B27 antibody clone GS145.2 or
an equivalent would improve the reliability of HLA B27 typing by
yielding results similar to those of PCR based assays.

Abstract 086

Pan Leuco Gating (PLG) CD4 Enumeration in Old Blood Samples: An Experience at a Reference Laboratory

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Background Flow cytometric enumeration of lymphocyte subsets in
older blood samples is difficult because of a poor separation of lym-
phocytes from the other leucocytes in scatter plots derived from these
samples. PLG CD4 reagents (M/s Beckman Coulter, USA) are reported
to yield more accurate CD4 counts in older blood samples compared to
other similar reagent combinations by virtue of a clear separation
between CD4 lymphocytes and monocytes. We examined the actual
performance of this method and compared the results with those of
another established, more expensive single-platform technology.
Materials and Methods EDTA and heparinised blood samples from 127
healthy adult males and 128 females were tested by two-colour PLG
CD4 reagents (CD45/SSC/CD4 sequential gating) and 20 of these were
tested in parallel by four-colour Tetrachrome reagents from the same
vendor (CD45/SSC/CD3/CD4/CD8 gating). Stability of the counts was
checked by both the methods at timed intervals in samples retained up to
5 days at room temperature. *Results* There was a high degree of cor-
relation between the CD4 counts obtained by PLG and by Tetrachrome
reagents ($r = 0.97$). Results from EDTA samples tested by PLG CD4
reagents showed a better stability (up to 48 h) compared to those
from heparinised samples (up to 24 h). The stability of the counts in
heparinised samples by PLG was significantly inferior to that by Tet-
rachrome (72 h). *Conclusion* CD4 enumeration by PLG CD4 reagents
is cheaper and is comparable in accuracy and reproducibility to more
expensive reagent panels. However, this technology appears less suit-
able for samples >48 h old, especially if collected in heparin.

Abstract 087

Leucocyte Adhesion Deficiency (LAD)-Type I. A Series of 13 Patients from a Single Tertiary Care Center in Southern India

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LAD-type 1 is a rare autosomal recessive disorder characterized by
defective leucocyte adhesion and chemotaxis, characterized by defi-
cient common subunit of β2-integrin CD18, As a result, normal
heterodimers of three α (CD11a, CD11b, and CD11c) and β sub-
units cannot form resulting in defective neutrophil function.

Immunophenotyping is an easily available test which is routinely performed for confirming the diagnosis of this disorder. The aim of this study was to describe the validation of a panel of antibodies for the diagnosis of type 1 LAD in our institute. LAD-type 1 patients, from 2005 to 2010 were retrospectively analyzed with regards to clinical features, haematological and ancillary laboratory parameters. Immunophenotypic data with regards to percentages and median fluorescence intensity (MFI) of CD11a, CD11b, CD11c and CD18 on the granulocyte population were analyzed in patients and normal controls. On gating normal neutrophils, the normal percentages for CD11b, CD11c, CD18 and CD11a were established first on normal controls and were found to be 99.1, 99.2, 98.9 and 92.3 respectively. Similarly the normal range of median fluorescence intensities on normal controls for CD11b, CD11c, CD18 and CD11a were found to be 821, 718, 620 and 411 respectively. Thirteen patients were detected to have type-1 LAD based on clinical and immunophenotypic criteria. In the patients the average percentages of CD11b, CD11c, CD18 and CD11a were 9.4 ($n = 12$), 0.3 ($n = 13$), 0.9 ($n = 12$) and 1.8 ($n = 13$) respectively and the respective MFIs were 344, 319, 323.5 and 348.5 for the aforementioned CD markers. Both the MFIs and percentages of CD11b, CD11c, CD18 and CD11a were decreased as compared to the normal levels. Based on the expression of CD18 (as a percentage), 12 out of the 13 patients could be classified as severe type 1 LAD (CD18 <1%), a single patient was of the moderate type (CD18–8.4%). The age of presentation ranged from 6 to 1,105 days (median: 120 days) with male predominant distribution (76.9% males). All patients had leukocytosis (mean 63.020/mm^3: range 34,400–86,900) and showed neutrophilia (mean = 76% neutrophils). Common infections included *Pseudomonas aeruginosa* (66.6%) and Staphylococcus aureus (50%). Two of the patients died while in the hospital. 80% of the patients had history of consanguinity and 66.6% had a family history of LAD [in all cases sibling(s) were affected]. 61.5% showed clinical features of omphalitis and all the patients had history of recurrent infections. This is a descriptive single center experience of a large cohort of patients with type 1 LAD from southern India. This disease could be consistently diagnosed and subclassified using a relatively simple panel of antibodies after validation on normal controls. As the percentages of CD11a, CD11b, CD11c and CD18 on neutrophils is markedly different from normal, generation of MFIs may not provide any additional information.

Abstract 088

Immunophenotypic Characterization and Quantification of Myeloid-Derived Suppressor Cells in Patients with Leukemia

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Myeloid-derived suppressor cells (MDSC) have recently been recognized as a distinct subset of the immune system that have significant immuno-modulatory properties. Importantly, they have been implicated to be associated with tumor immune escape mechanisms. Growing evidence suggests that MDSCs or immature myeloid cells play a crucial role in the progression and persistence of cancer in tumor bearing mice and in cancer patients (Cancer Immunol Immunother, 2009). In mice this subset is phenotypically characterized as CD11b$^+$

Gr1$^+$ cells while in humans they have a phenotype which is CD14$^-$ HLADR$^-$ CD15$^+$ CD33$^+$ and CD11b$^+$ (Nature Rev Immunol, 2009). There is limited data on the presence of this subset and their prognostic significance in acute leukemia. We undertook a prospective study at our centre from June, 2010 to August, 2010 on peripheral blood samples of healthy controls ($n = 8$) and on newly diagnosed leukemia patients (AML = 21, Pre-B ALL = 12, Tcell ALL = 4, APL = 10) to investigate the presence of MDSC subsets using flow cytometry. To detect MDSCs, peripheral blood mononuclear cells were isolated and labeled with a panel of monoclonal antibodies to CD14, CD33, HLADR and CD33 directly conjugated with FITC, PE, PerCP and APC respectively and incubated for 20 min. Cells were then washed and analyzed using FACSCalibur and CellQuestPro software. 100,000 events were collected for all analysis. Acquired cells were first gated (R1) based on FSC vs. SSC. The second gate (R2) comprised of CD14$^-$ HLADR$^-$ cells. From this population, the fraction of cells expressing the myeloid marker CD33 and CD11b was determined (R3, summarized in Fig. 1). MDSC for this study was hence defined as CD14$^-$ HLADR$^-$ CD33$^+$ CD11b$^+$ (modified from J Immunol, 2009). The median number of MDSC in peripheral blood samples of controls and newly diagnosed AML, Pre-B ALL, TALL and APL was 0.68% (range 0.11–0.97), 0.28% (range 0.01–11.55), 0.15% (range 0.01–1.47), 0.13% (range 0.02–0.27) and 0.36% (range 0.04–1.54) respectively. The median values were significantly lower in ALL vs. AML ($P = 0.05$). There was significant heterogeneity in the proportion of this subset in cases with a diagnosis of AML as illustrated in Fig. 2. Seven of 21 (33.3%) AML cases had a MDSC subset that was more than twice that of controls and ranged from 1.18 to 11.55%. In these patients with a high MDSC percentage there was no correlation with WBC counts at diagnosis, cytogenetics or molecular markers such FLT3 and NPM1 mutations. In conclusion this preliminary analysis, the first to study this subset in leukemia, suggests that a significant population of MDSC are seen in a proportion of patients with AML but very rarely seen in other acute leukemia's. Long term follow up data is required to see if the presence of a significant number of this subset at diagnosis in patients with AML has a prognostic significance as has been reported with some solid tumors.
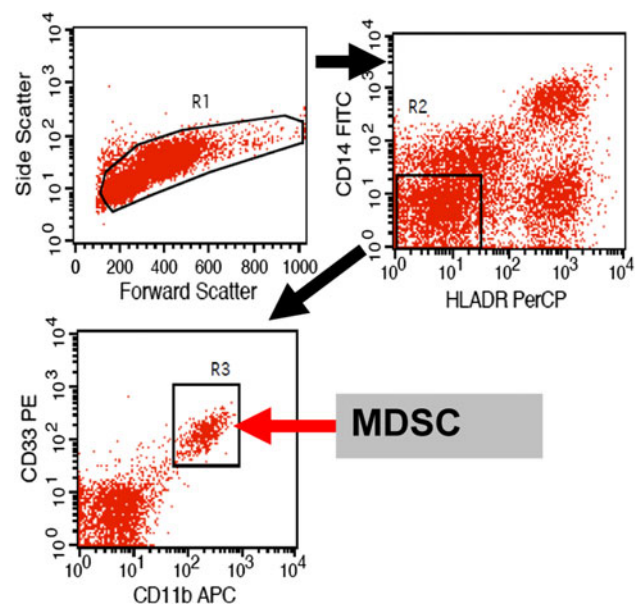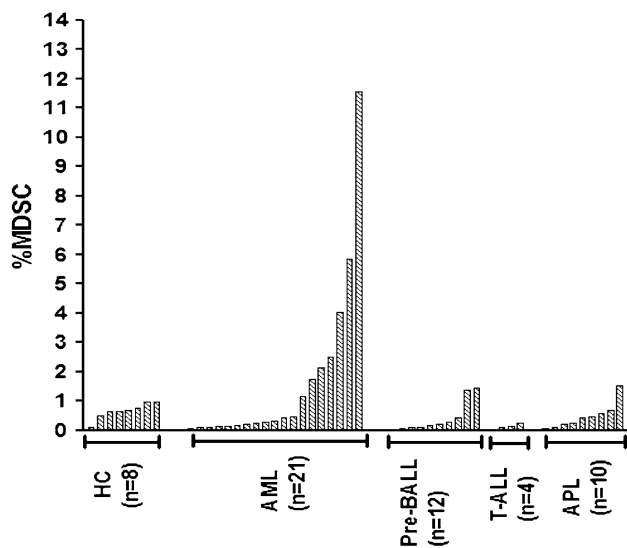

Fig. 1 Gating strategy for MDSC

Fig. 2 Percentage MDSC in different subsets of acute leukemia

Abstract 089

Stromal Interaction has an Adverse Impact on In Vitro Sensitivity of NB4 Cell Line to ATO and can be Overcome by use of Bortezomib and Lenalidomide at Pharmacologically Relevant Concentrations

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Arsenic tri-oxide (ATO) has proven efficacy in the treatment of acute promyelocytic leukemia (APL). However, some newly diagnosed and a significant proportion of relapsed patients with APL relapse following therapy with an ATO based regimen. There is limited data on the impact of stromal interaction on malignant promyelocytes. Stromal interaction with acute myeloid cells has been reported to lead to drug resistance; it is possible that a similar protective effect against ATO could be seen with APL cells. To study this we used a malignant promyelocytic cell line (NB4) and normal mesenchymal stromal cells (MSC). Bone marrow samples were obtained from patients who had a bone marrow done for a non malignant haematological condition after getting informed consent. MSC were expanded from these samples using previously established methods and only MSC from 1st or 2nd passage was used for these experiments. We standardized an in vitro IC-50 assay using an established MTT viability kit. Cell viability post incubation with drugs evaluated was also studied by flow cytometry using 7AAD and annexinV staining. Clinical grade (>99% purity), non formulated bortezomib (Bo) and lenalidomide (Le) were obtained (gift from NATCO pharma) and a working solution prepared by re-suspending in DMSO. This solution was further diluted in appropriate media for experiments (final DMSO concentration <0.00001%). The mean IC-50 for NB4 cells with ATO alone was 0.93 µmol ($n = 8$). The mean IC-50 of NB4 cells with Bo was 9.8 ng/ml ($n = 5$), the pharmacological levels achieved in vivo with conventional dose of this drug ranges from 20 to 106 ng/ml. Le at pharmacologically relevant doses did not appear to kill NB4 cells. There was no significant MSC kill over the range of doses used in these experiments with

ATO, Bo or Le. IC-50 values of NB4 cell to ATO was significantly reduced when combined with Bo over a wide range of pharmacologically relevant concentrations of this drug (Fig. 1). Combination of ATO with Le however did not change the IC-50 value of NB4 cells to ATO. Co-culture of NB4 with MSC and exposure to ATO for 48 h at 1, 2, 4, 6 and 8 µmol concentrations consistently resulted in a significant increase in NB4 cell viability compared to NB4 exposed to ATO without MSC ($n = 5$). A similar protective effect (less robust) was also seen when MSC conditioning media was used alone (data not shown). Combining ATO and Bo or ATO and Le, over a wide range of pharmacologically relevant doses of Bo and Le, significantly reduced the NB4 cell viability compared to the value obtained with ATO alone on co-culture with MSC. Figure 2 illustrates the impact of ATO at 2 µmol in combination with different concentrations of Bo and Le. This is the first report demonstrating the protective effect of stromal interaction on the effect of ATO on a promyelocytic leukemia cell line. We have also demonstrated the potential for both Bo and Le to synergize with ATO and over come this protective effect and the independent effect of Bo on NB4 cells. Further studies are required to establish the mechanism by which this protection is mediated and those by which Bo and Le are able to overcome them.
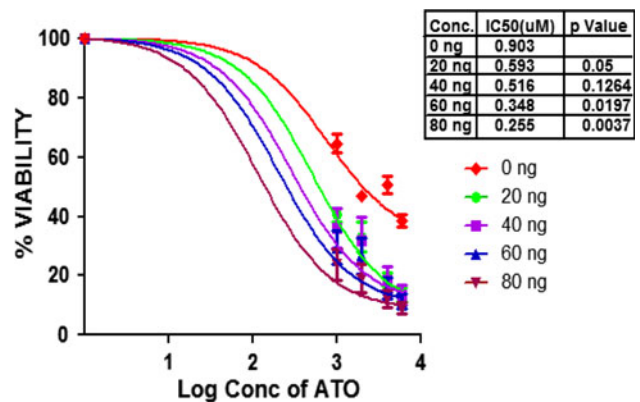

Fig. 1 Combined effect of ATO and Bo (different concentrations) on IC-50 value of NB4 cells to ATO
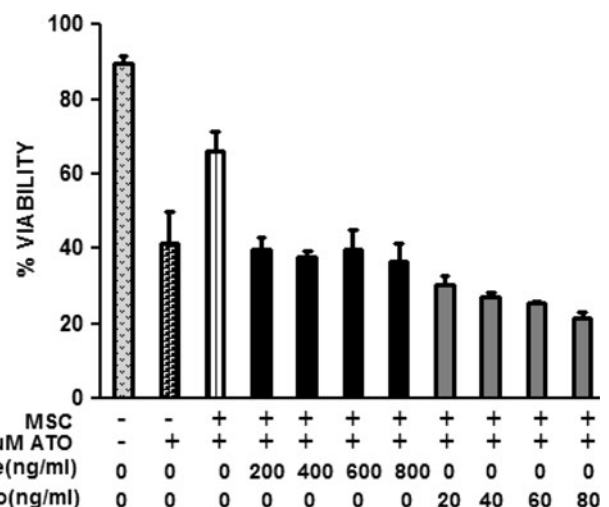

Fig. 2 Protective effect of stromal cells on NB4 and the synergistic effect of lenalidomide and bortezomib along with ATO on these interactions (48 h incubation)

Abstract 090

Prognostic Correlation of Very Late Activation (VLA) Antigens VLA-4 and VLA-5 on Leukemic Blasts at Diagnosis and Leukemia Initiating Cell Quantity at Diagnosis and Day +14 of Induction in a Cohort of Patients with Acute Myeloid Leukemia

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The interaction of leukemic blasts with the bone marrow (BM) microenvironment is postulated to be mediated predominantly by the $\beta 1$ integrins (VLA-4 and VLA-5), present on the blast surface. This phenomenon is known to account for minimal residual disease due to blast adherence to BM stroma, resulting in resistance to chemotherapy (Nat Med, 2004). We also hypothesized that the quantification of leukemia initiating cell (LIC), population at diagnosis and on a day 10–14 bone marrow to assess response to therapy should have prognostic significance. Prospective quantification of VLA-4 and VLA-5 expression at diagnosis in 39 patients with non M3 AML (Oct., 2008–Dec., 2009) was evaluated by immunophenotyping (IPT) along with percentage of LIC populations (CD34+CD38−) at diagnosis and day +14. Standard variables like cytogenetics, type of induction, type of treatment (transplant, chemotherapy) and MFI of CD34 at diagnosis were also evaluated against overall (OS), event free survival (EFS) and incidence of relapse. The median age of the patients was 36 years (range 5–59 years) and there were 61.5% males. By karyotyping there were 3 (7.5%), 30 (77%) and 6 (15.5%) patients in the good, intermediate and high risk subsets respectively. The median fluorescent intensity (MFI) of VLA-4, VLA-5 and CD34 at diagnosis was 201.69 (range 44.11–537.61), 201.69 (range 36.85–842.91) and 89.77 (range 29.16–486.97) respectively. The median LIC value at diagnosis and on a day 10–14 bone marrow was 0.1 and 0.05% respectively. There was no apparent correlation with expression of these markers at diagnosis with age, WBC count and blast index at diagnosis, or cytogenetic risk groups. The 1 year KM-estimate of OS and EFS of this cohort 70.95 $\pm$ 7.96% and 60.10 $\pm$ 8.43% respectively. On a uni-variate cox-regression analysis a high MFI of CD34 was associated with a significant adverse impact on overall survival ($P = 0.04$), while a high MFI of VLA-5 was associated with a trend towards reduced survival ($P = 0.06$). Similarly a high MFI of CD34 at diagnosis was also associated with a trend to a lower EFS ($P = 0.074$). LIC percentage at diagnosis did not correlate with OS or EFS. However, a day 14 LIC percentage was associated with a significant increased risk of relapse (RR = 2.59 [1.094–6.13]; $P = 0.03$). Using the day 14 median LIC value as a cutoff resulted in identifying a subset with trend to inferior overall survival ($P = 0.07$) (Fig. 1). These preliminary observations have the potential to identify immunophenotypic markers that could serve as inexpensive prognostic markers in AML. They however remain to be validated in a larger cohort with longer follow up.
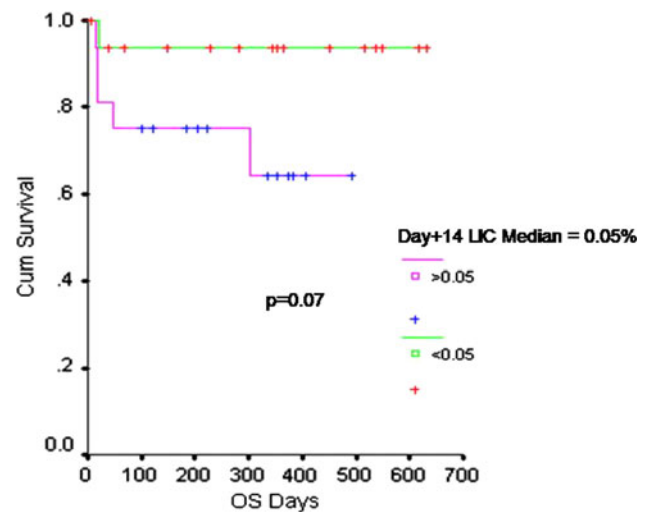

Fig. 1 KM estimate of overall survival above and below the median LIC value on a day 10–14 bone marrow sample

Abstract 091

Hypobaric Hypoxia Induces Endothelial Dysfunction and Haemostatic Imbalance

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High Altitude (HA), with its attendant hypobaric and cold condition results in certain stresses to the human body. Rapid ascent of the climbers to extreme altitude has been found to induce major thrombotic episodes such as deep vein, cerebral and portal vein thrombosis. Long stay at HA leads to an increase in the propensity of thrombosis (with a thirty times higher incidence) seen in Indian soldiers. High altitude can precipitate deep vein thrombosis because of hypobaric hypoxia induced activation of coagulation system especially due to the disruption of haemostatic balance. Dehydration, polycythemia, and vascular spasms may also be involved in the increased tendency to venous thrombosis at high altitude. Vascular endothelium plays a major role in maintaining the normal haemostatic balance by releasing different pro-coagulant/anti coagulant molecules. Endothelial cell injuries and resultant endothelial dysfunction plays a key role in all types of thrombotic disorders. Vascular endothelium which is strategically located at the interface between tissue and blood is primarily affected by systemic hypoxia. Hypoxia induced endothelium injury changes the expressions of pro-coagulant/anti-coagulant molecules in the sub-endothelial matrix that tilts the haemostatic balance towards thrombosis. Dysfunction of endothelium allows platelets to adhere to the sub-endothelial surfaces, resulting in activation and release of pro-coagulant molecules like Von Willebrand Factor (vWF), Adenosine Diphosphate (ADP) and Thromboxane A2 (TXA2). These molecule recruits additional platelets and amplify

platelet activation and aggregation. *Aim of the Study* Characterization of pro/anti coagulant molecule and their role in hypobaric hypoxia induced hypercoagulable state. Study of hypoxia induced endothelium dysfunction and inflammation that leads to thrombosis. *Methodology* 12 male Sprague–Dawley rats were randomly selected and divided into four groups. One group was kept as control and the other three groups were exposed to hypobaric hypoxia for 6, 12, and 24 h respectively. Human Umbilical Vein Endothelial cell (HUVEC) was also exposed to hypoxia for similar time period. The expression of Tissue Factor (TF), Phospho Disulfide Isomerase (PDI), vWF, Vascular Cell Adhesion Molecule (VCAM), Intracellular Adhesion Molecule (ICAM), P-Selectin, E-selectin, Thrombomodulin (TM), Tissue Factor Pathway Inhibitor (TFPI) Interferon alpha (IFN-α) were measured by western blot and ELISA. Prothrombin time (PT), activated partial thromboplastin time (APTT), and Fibrinogen level were measured by standard operation procedure of ST art® 4 Haemostasis analyzer (Diagnostica Stago). *Results and Discussion* Hypoxia affects endothelial cellular physiology in various ways and regulates the synthesis and release of vasoactive substances depending on the degree and duration of hypoxia. Endothelium is a dynamic tissue that acts as an active barrier between the vascular lumen and tissues. In our study we found that HA hypoxia initially induces TF expression which returns to the basal level in 24 h of continuous hypoxia. The expression of vWF however increases gradually throughout the time of hypoxia exposure. The expression of endothelial dysfunction markers like VCAM, ICAM, E-Selectin, P-Selectin, PDI, IFN-α increase due to hypoxia exposure. On the other hand the expression of anti-coagulant molecule like TM and TFPI goes down upon hypoxia exposure. *Conclusion* According to our findings so far, we can conclude that hypoxia causes endothelial dysfunction, upregulation of pro-coagulant molecule and downregulation of anti-coagulant molecules.

Abstract 092

A Randomized Comparison of Early Versus Delayed Administration of G-CSF in High Dose Cytarabine Consolidation Chemotherapy of Acute Myeloid Leukemia

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Introduction Granulocyte colony stimulating factor (G-CSF) is usually used after high dose cytarabine (HIDAC) consolidation chemotherapy of acute myeloid leukemia. However optimal time of starting G-CSF is not clear. Delayed administration of G-CSF may be as effective with the benefit of substantial cost saving. *Objective* A prospective randomized comparison of delayed (D + 12) versus early (D + 7) administration of G-CSF after high dose cytarabine consolidation chemotherapy of acute myeloid leukemia. End points were duration of neutropenia, incidence of neutropenic fever, fungal infections and short term mortality. *Materials and Methods* All the patients of acute myeloid leukemia, being treated with high dose cytarabine (3 gm/m^2/dose q 12 h on day 1, 3 and 5) were randomized in two G-CSF (dose 5 µg/kg/day/SC) groups; group A (D + 7 of chemotherapy) and group B (D + 12 of chemotherapy), which was continued till recovery of absolute neutrophil count (ANC) >1,000/µl. Oral levofloxacin and fluconazole was used for prophylaxis. Neutropenic fever was treated as per standard guidelines and HRCT was used to diagnose fungal infections. *Results* Total number of HiDAC cycles analyzed 88 (group A 42, group B 46), both the G-CSF groups were comparable to each other for age, sex, distribution in different

HiDAC cycle, presenting cytogenetics, WHO subtype and antifungal prophylaxis. Median duration of neutropenia 13 days (group A 12, group B 13; *P* 0.15), neutropenic fever 62.5% (group A 66.7%, group B 58.7%; *P* 0.582), median duration of thrombocytopenia 19 days (group A 20, group B 19; *P* 0.66), CT evidenced fungal infections (group A 9.5%, group B 8.6%; *P* 1.0), mortality (group A 4.8%, group B 4.3%), and side effects of bone pain were not different between two randomized groups. Statistically significant difference was seen in median days of G-CSF use (group A 14, group B 10; *P* <0.001) which translated in cost saving of Rs. 8,000/HiDAC cycle and lesser number of painful injections given to patients. *Conclusion* G-CSF administration after HiDAC consolidation can be delayed by 5–6 days after completion of chemotherapy without compromising clinical benefit while saving substantial cost which is welcome in a resource poor country like India.

Abstract 093

Platelet Related Bio Marker of ACS

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Background An *acute coronary syndrome* (ACS) is a complex disease, and becoming the predominant disease of death in the world. Platelet hyper-function contributes to acute coronary syndromes (ACS). But till now there no platelet related genetic marker for ACS. *Objective* The objective of this work is to find out any platelet related genetic marker. *Method* Two SNPs in P2Y1 and P2Y12 gene are identified for the genotyping in the eastern Indian population for ACS. Both the SNPs are silent. Total study is carried out on 25 normal healthy individuals and 25 ACS patients. We have also study the smoking habit and presence of hypertension among these two groups. *Results* it was found, among the two groups of individuals ($n = 50$), two ACS patients carry the P2Y12 polymorphism. From the statistical analysis we have also found that cigarette smoking added 4.9 times extra risk for ACS. *Conclusion* from the results it can be concluded that ACS patients have polymorphic site on P2Y12, so that silent mutation may have any intrinsic role for ACS, and smoking is an important risk factor for ACS.
Keywords Platelet, ACS, P2Y12, P2Y1

Abstract 094

Spectrum of Bacterial Infections Encountered During Management of Acute Leukemia Patients

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Background Bacterial infections are a major cause of complications and death in patients with haematologic cancers and chemotherapy induced neutropenia. Over the last three decades, there has been a

significant change in the spectrum of infections in neutropenic patients with acute leukemia. With the introduction of beta-lactamase-resistant antistaphylococcal penicillins, gram-negative bacilli became the predominant bacterial organisms including *Escherichia coli*, Klebsiella species and *Pseudomonas aeruginosa*. The Enterococcal species, *E. faecalis* and *E. faecium* have emerged as virulent pathogens due to the acquisition of antibiotic resistant plasmids. We present bacteriological data from our acute leukemia inpatients from an institute which is a referral center for benign and malignant hematological disorders in the setting of a tertiary care hospital in Kolkata. *Aim* To study the emerging spectrum of bacterial infections and antibiotic sensitivity pattern in acute leukemia patients undergoing treatment in a general ward. *Methods* We gathered data on all acute leukemia inpatients managed between 1st August 2008 and 30th November 2009. Rapid culture by BacT/Alert 3D system (Biomerieux) was performed in all samples. All episodes of fever are considered to be due to infection, unless proved otherwise and blood cultures are sent routinely at the onset of fever and repeated as and when necessary. The central venous catheter tip is also sent for bacterial culture when it is removed, if indicated. Empiric antimicrobial therapy was initiated in all febrile patients. The gram-positive coverage added to the first line empiric antibiotic therapy as per suspicion. Anaerobic coverage and antifungals, either as empiric/preemptive, was instituted as per institutional policy. Antibiotic prophylaxis with levofloxacin for all neutropenic patients was routinely prescribed. Patients with ALL also received cotrimoxazole for prophylaxis against *Pneumocystis jiroveci*. *Results* A total of 202 blood cultures were sent during the above period, and bacterial isolates were documented in 63 cases (31.2%). Gram negative organisms constituted 61% (38/63) and Gram positive organisms 39% (25/63). Gram positive infections—Gram positive isolates were Staphylococcus (*Staphylococcus aureus* and coagulase negative staphylococcus) in 84% cases. The remaining 16% of Gram positive isolates were Enterococci sensitive to Vancomycin. There were more Methicillin-resistant (MRSA) strains (53%) than Methicillin-sensitive S aureus (MSSA). 67% of the Coagulase negative Staphylococci were methicillin-sensitive. Gram negative infections—*Pseudomonas aeruginosa* was the commonest Gram negative bacteria causing febrile neutropenia (31%), followed by *Escherichia coli* (18%), *Klebsiella pneumoniae* (13%) and *Acinetobacter baumannii* (13%). Enterobacter species was isolated during two episodes, and *Proteus mirabilis* was isolated in 1 episode. Multi drug resistant organisms—Majority of the Enterobacteriaceae (83% isolates) demonstrated Extended Spectrum Beta Lactamase (ESBL) activity. There was very high rate of fluoroquinolone resistance among the Gram negative bacteria isolated. Multidrug-resistant *Pseudomonas aeruginosa*, sensitive only to Polymyxin B and Colistin, was isolated from a terminal patient with relapsed ALL. Uncommon bacterial isolates—There were 2 isolated reports showing growth of *Stenotrophomonas maltophilia* associated with febrile episodes. The Stenotrophomonas isolates were resistant to carbapenems in all the cases. All the seven patients responded to treatment with cefoperazone + sulbactam and amikacin. Fungal isolates—In addition to the bacterial isolates, non-albicans Candida species were isolated from non-tunnelled central venous catheter (CVC) blood samples from three patients and Candida albicans from 1 patient, during episodes of febrile neutropenia. The non-albicans Candida species isolated included *C. tropicalis*, *C. krusei* and *C. globosa*. Also, fluconazole resistant *Saccharomyces kluvverii*, a true yeast, was grown from consecutive CVC blood samples of two patients with febrile neutropenia, requiring CVC removal in one patient; the CVC tip culture yielded significant growth of the same organism. *Conclusion* Our brief analysis of the emerging spectrum of bacterial infections confirms predominance of gram-negative bacilli in acute leukemia patients. With the widespread antibiotic prophylaxis with levofloxacin for all neutropenic patients, there is emergence of fluoroquinolone resistance among the bacteria isolated.

Abstract 095

An Approach to Pediatric Thrombosis in Under Resourced Laboratories: A Report of 229 Cases from India

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Retrospective analysis was conducted on 229 pediatric patients with arterial (144) and venous (85) thrombosis referred to hematology department at All India Institute of Medical Sciences for prothrombotic work up. ProC global was used as an initial screening test. The children with low ProC global were screened further for various inherited defects, protein C (PC) deficiency in 21 (32.2%), protein S (PS) deficiency were found in 12 (20.3%). Amongst children who were screened for APCR, a total of 59 only 2 (5%) were positive. The combined deficiency of PC and PS was found in eight children screened and four had abnormal AT III levels. Beta-2-GPI was tested in 50 suspected children in whom three were positive (6%). Two cases revealed raised serum Homocysteine levels. Molecular studies of factor V G1691A defect was seen in only two cases whereas MTHFR C667T polymorphism was not found. In children with arterial thrombosis protein C deficiency was found in 32%, protein S deficiency in 20.5%, 5.1% had low ATIII, combined protein C and S deficiency in 17% children, raised beta-2-GPI levels in 8% cases. Serum homocysteine and factor V leiden mutation were found in two cases each?) Children with venous thromboembolism 50% had protein C deficiency, 16.6% had protein S deficiency and ATIII deficiency was seen in 11%. In 1 child Factor V leiden mutation was demonstrated. Among these, 2 (16%) children had combined protein C and protein S deficiency. In 3.4% Beta-2-GPI levels were raised. Protein C system defects are the commonest inherited causes of thrombosis in the pediatric age group in India. A stepwise approach in testing children to detect the underlying deficiency will be helpful to reach the final diagnosis in under resourced laboratories where financial constraints play a major role.

Abstract 096

Managing Aplastic Anemia in India: ATG Plus Cyclosporin Versus Cyclosporin Alone

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Background When compared to the West, aplastic anemia is much more common in India. A response rate to anti thymocyte globulin and cyclosporine (ATG + CSA) is around 80% in the west where as the Indian studies shows response around 50–60% which is comparable to cyclosporine (CSA) alone. There is no prospective study in India which directly compares ATG + CSA with CSA alone. This study compares the response to ATG + CSA and CSA alone in aplastic anemia patients. *Aim* to compare response to treatment with Cyclosporine alone versus the combination of Antithymocyte Globulin and Cyclosporine in patients with aplastic anemia to determine whether CSA alone is as effective as the combination of ATG and CSA, which is the current best immunosuppressive treatment for acquired aplastic anemia. *Methodology* Diagnosed patients of aplastic anemia who were not suitable or unable to afford bone marrow transplantation without any active infection were included. Thirty-one patients were enrolled in the study. Twenty patients were given CSA and eleven patients were given ATG followed by CSA. All patients

were followed up once in 2 weeks, during each follow-up, Hb, TLC, ANC, platelet count were done to assess the response and urea, creatinine and electrolytes monitored regarding the occurrence of side effects. A response to therapy was considered if there was an increase in baseline Hb, TLC, platelet count and decreases in the requirement of blood transfusions as compared to the state before starting therapy. *Results* Three patients in ATG and CSA group died within 2 months of therapy and four patients discontinued therapy in CSA group, 24 patients were finally analyzed. After 3 months of therapy, 9 out 16 (56.5%) in CSA group and 5 out of 8 (62.5%) in ATG and CSA group responded to therapy. *Conclusions* CSA mono therapy is as efficacious as combination with ATG. In developing countries like India; CSA is a reasonable therapeutic option.

Abstract 097

Clinical and Hematological Profile of Hb SD-Punjab in Nine Families of North India

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Background HbSD-Punjab is a rare hemoglobinopathy. This compound heterozygous state is a combination of Hb sickle and HbD-Punjab. Not much is known about its severity and clinical course. *Objectives* To study the clinical and hematological profile of compound heterozygous HbSD-Punjab in North Indian patients. *Methods* HbSD-Punjab patients registered in our pediatric and adult hematology clinics from 1995 to 2010 were enrolled. Data of their clinical profile was retrieved from medical records and analyzed. *Results* A total of 10 patients (9 families) were identified. It comprised of six males and four females. The median age at onset of symptoms and diagnosis were 4.7 (range 0.7–10) and 8.2 years (range 1.1–19) respectively. Their mode of presentation varied; two (20%) with plasmodium vivax malaria, two (20%) with acute chest syndrome, two (20%) with painful vaso-occlusive crisis and remaining four (40%) with anemia. All had moderate to severe anemia (mean Hb 7.5 ± 1.9 gm/dl). Seven (70%) required red cell transfusion [median 3 (2–12)]. The median age at first transfusion was 7 years (1–19). Jaundice, hemolytic facies, growth retardation (Height <3rd centile) and splenomegaly were present in 6 (60%), 3 (30%), 7 (70%), 4 (40%) cases respectively. The diagnosis was established by HPLC, Hb electrophoresis, molecular analysis and family screening. On follow up, one of them developed avascular necrosis of femoral head and three had recurrent painful vaso-occlusive episodes. *Conclusions* HbSD-Punjab has a heterogeneous clinical presentation. Anemia and sickle crises are quite common. This compound heterozygous state is the only condition with HbD Punjab which leads to moderate thalassemia intermedia phenotypically.

Abstract 098

Molecular Analysis of Genes Involved in Hereditary Haemochromatosis in North Indian Patients

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Introduction The *HFE* and non-*HFE* (*TFR2, HJV, HAMP* and *Ferroportin*) genes associated with iron overload were identified in patients with cryptogenic chronic liver disease (CLD). *Objective* To establish mutational spectrum of *HFE* and non-*HFE* (*TFR2, HJV, HAMP* and *Ferroportin*) genes in north Indian patients with Hereditary Haemochromatosis (HH). *Methods* A total of 238 patients with CLD were screened for iron status (TIBC, %TS, serum iron and ferritin) to identify cases with HH. DNA was extracted using standard phenol–chloroform method. The *HFE* and non-*HFE* (*TFR2, HJV, HAMP* and *Ferroportin*) genes were analysed by PCR–RFLP and DNA sequencing. The frequency of observed mutations/polymorphisms was also determined in control samples. *Results* The overall prevalence of primary iron overload in CLD patients was 7.1%, out of which 18 were confirmed cases of primary iron overload. The two mutations in *HFE* gene, namely C282Y and S65C were absent in our population. However, the prevalence of H63D was similar in 238 CLD patients and 100 normal individuals i.e. 14.2 and 12%, respectively. Sequencing of *HFE* showed only one case with a mutation i.e. T217I of *HFE* gene; this was compound heterozygous for H63D and T217I of *HFE* gene. IVS2+4, IVS4−44, IVS4+47, IVS5−47 were the observed polymorphisms in *HFE* gene. We identified two patients to be homozygous for a novel protein truncating mutation in *HJV*, at 336 G (1006 G→T results in 336X) which results in a premature stop codon. The *TFR2, HAMP* and *Ferroportin* genes did not show any mutation. *Conclusion* HH is an uncommon disorder in the Indian subcontinent and the causative mutation is different from the cases of HH in the west.

Abstract 99

Evaluation of Mechanisms of Resistance to Arsenic Trioxide in Patients with Acute Promyelocytic Leukemia

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Introduction Newly diagnosed and relapsed acute promyelocytic leukemia (APL) patients respond to therapy with arsenic trioxide (ATO) based regimens. Significantly more patients with relapsed APL have disease recurrence after ATO based therapy than newly diagnosed cases. We undertook a series of experiments to evaluate the potential mechanisms to explain this increased risk of relapse in patients with relapsed APL. *Patients and Methods* From April 2007 to March 2009 bone marrow samples from newly diagnosed and relapsed cases admitted at our center were utilized for these studies. If required the bone marrow blasts and promyelocyte component was enriched to above 90% using a lineage depletion cocktail and a VarioMACS (Miltenyi Biotec, Germany). For in vitro intracellular ATO concentration measurement, 2×10^7 cells were washed and suspended in RPMI media with 0.5 µM concentration of ATO and incubated for 24 h. Cells were then washed, made into a pellet and solubilized with HNO_3 and H_2O_2 and ATO content measured using an atomic absorption spectrophotometer. An in vitro sensitivity assay of malignant cells as previously reported was standardized using an MTT assay system. The impact of co-culture of mesenchymal stromal cells (MSC) and malignant promyelocytes on ATO induced apoptosis was studied with 7AAD and Annexin staining using a flowcytometer. A gene expression array using 44k human microarray chip analysis (Agilent technologies) was done on eight newly diagnosed and eight relapsed cases. *Results* Sixty-five patients were included in this study. Of these 47 (72%) were newly diagnosed and 18 (28%) were relapsed cases. On immunophenotyping CD34 was positive (>20%) in 3.6% of newly diagnosed and 50% of relapsed cases ($P = 0.001$). The mean MFI of the relapsed

cases for expression of CD38, VLA-5 and CD13 was significantly lower in the relapsed group. The ability of both newly diagnosed and relapsed primary APL cells to concentrate ATO intracellular was not significantly different (15.2 ± 9 nG/10^7 cell vs. 16.3 ± 9.7 nG/10^7 cell). Similarly the in vitro IC-50 assay was not significantly different between the two groups (5.5 ± 3.8 vs. 4.7 ± 4.5 μM). Neither did either of these assays correlate with clinical parameters such as relapse, event free (EFS) or overall survival (OS). Evaluation of effect of MSC on ATO demonstrates a protective effect of MSC on ATO induced apoptosis both in newly diagnosed and relapsed cases (Fig. 1). This effect was mediated partly by the MSC conditioning media and could not be overcome by addition of VLA-4 or VLA-5 blocking antibodies (data not shown). Gene expression studies comparing the two cohorts revealed 1744 genes that were differentially expressed (>2 fold) between samples at diagnosis and at relapse. Quantitative Real-time RT–PCR using SYBR-Green detection system was done to confirm the gene expression results obtained from microarray analysis. Using ΔΔCT method the fold difference was calculated for five selected genes which validated the microarray data. *Conclusion* Relapsed patients have significant immuno-phenotypic differences from newly diagnosed cases. Mechanisms of resistance to ATO are probably multi-factorial but are unlikely to be related to intra-cellular ATO concentration. IC-50 does not appear to predict clinical outcomes. Stromal interaction appears to protect malignant promyelocytes from the apoptotic action of ATO which appears more pronounced in relapsed than in newly diagnosed cases. Further evaluations of parameters that enhance such stromal interaction and protect malignant promyelocytes from the apoptotic effect of ATO along with evaluation of dysregulated genes and pathways are required.
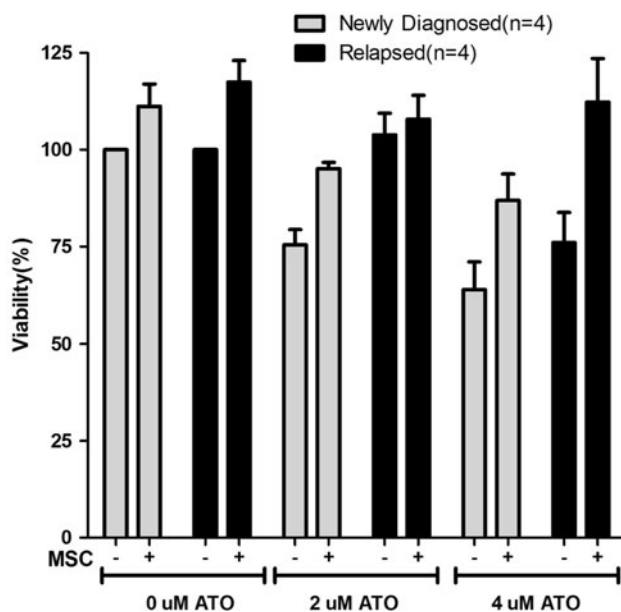

Fig. 1 Effect of stromal interaction on effect of ATO on malignant promyelocytes and blasts from newly diagnosed (*n* = 4) and relapsed patients (*n* = 4) with APL. The bar graph is normalized such that newly diagnosed patients cells not exposed to ATO should have 100% viability after 48 h incubation

Abstract 100

Data Mining to Identify the Contributions of Copy Number Variations in Altered Gene Expressions in Chronic Myeloid Leukemia: Preliminary Experimental Validation

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Among the various causes of alterations of gene expressions in different disease including cancers, copy number variation (CNV) may play an important role. CNV defines as the gain or loss segment of DNA that varies between two homologous chromosomes. Genes residing within such variable segment may result in the variations in gene expressions due to alteration in the number of copies of the segments. To identify if CNV contributes towards the variations in gene expressions among the chronic myeloid leukemia (CML) patients, we down loaded altered gene expression data from Onomine database (https://www.oncomine.org/), a cancer microarray database for CML. Two thousand eight hundred thirty-nine genes are up regulated and 4453 genes are down regulated. Chromosomal locations (gene co-ordinates) of all the up-regulated and down-regulated genes were determined using BIOMART system (linked through ensemble genome browser) which is query oriented data management system developed jointly by Ontario Institute for Cancer Research (OICR) and the European Bioinformatics Institute (EBI). For each of chromosome, frequencies of the genes were calculated with in 1 Mb window. These frequencies of the genes within 1 Mb regions are then plotted against the distance from the tip of the chromosome. We observed marked differences in the frequency of genes in up regulated from the down-regulated genes in a particular stretch of seven chromosomes. It is to be mentioned that the expression of a particular gene varies widely from individual to individual as well as various stages of the disease. This observation prompted us to hypothesize that the genes residing with in particular CNV region could be the cause of such variations in the gene expressions. From the human genome variation database (http://projects.tcag.ca/variations), we then mapped the CNV loci on to the variable gene expression region. Thirteen genes were selected from these seven chromosomes, which are also located in different CNV loci. Primers were designed from these genes. We then determined whether this CNV regions are implicated in CML. Initial result with 85 CML patients and 30 control DNA samples, obtained from peripheral blood reveals variations in the DNA level in three out of thirteen CNV loci. Results will be presented and implications will be discussed.

Abstract 101

Plasma Proteomics in Hematological Disorders

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Purpose Hematological disorders can be classified into three main categories. Firstly, the red blood cell related disorders, such as thalassemia and sickle cell disease. Secondly the white blood cell related disorder such as the leukemia and thirdly the platelet related disorder such as thrombocytopenia and hemophilia. We are aiming to identify through plasma proteomic study a set of plasma proteins whose differential regulation is unique to only HbEβ-thalassemia. In HbEβ-thalassemia, HbE (HbEβ-26 Glu→Lys) interacts with

HbEβ-thalassemia to produce clinical manifestation of varying severity. Although HbE does not result in clinical severity, its combination with HbEβ-thalassemia increases the pathophysiological severity of the disease to varying phenotypes ranging from severe transfusion dependent thalassemia major to thalassemia intermedia that mainly include ineffective erythropoiesis, faster aging and premature hemolysis of the red blood cells. *Experimental design* For this study we are using 2D-gel electrophoresis and tandem MALDI mass spectrometry based techniques to investigate the differential proteome profiling of the plasma of normal samples, HbEβ-thalassemia, another erythrocyte related disorder (sickle cell disease), and a non erythrocyte related disorder (ALL). We are further validating the data by differential in gel electrophoresis (DIGE) and western blotting. *Conclusion and Clinical Relevance* We have observed interesting changes in the proteome of the plasma samples of different hematological disorders. The result obtained from this study might shed some light on the key players in the plasma which are responsible for the pathophysiology of HbEβ thalassemia.

Abstract 102

Platelet Proteomics in Haematological Disorders

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Platelets play important roles in fundamental biological processes including thrombosis, inflammation, wound repair and stroke. Clinical proteomics studies have been aimed for profiling of platelet proteins in healthy versus pathological states to discover specific marked expression changes for early diagnosis and disease progression using high resolution LC based and 2D Gel based protein separation and advanced mass spectrometric techniques to identify and characterize the proteins. Defective platelet aggregation has been reported in response to agonists in β-thalassemia major patients and associated with high platelet counts. The anomalies have been interpreted as signs of mild bleeding disorder because many thalassemic patients experience frequent epistaxis as well as easy bruising. The presence of morphologic platelet abnormalities in patients with β-thalassemia/hemoglobin E disease may also contribute to an enhanced risk of vascular complications. *Objective* To study the changes in proteome of platelets both in normal and in hematological disorders by the proteomics approach. *Methodologies* The strategy for platelet proteomic analysis incorporates platelet isolation as the initial step. Blood is collected from individuals in Acid Citrate Dextrose and platelets are resuspended in Tyrrodes HEPES buffer. Platelet count is recorded from Hemocytometer and purity of platelet preparation is checked by flow cytometric identification with CD41a antibody. The platelets are lysed; the lysate is run in 2D gel electrophoresis and stained. The spots are excised and subjected to tryptic digestion followed by identification by MALDI tandem mass spectrometry. *Results and Conclusion* The study of the platelet proteomics has just been initiated. Isolated platelets were ∼90% pure, as determined by flow cytometry. Nearly 200 spots have been visualized from the 2D Gel electrophoresis of the protein lysate. The 2D gel profile obtained has been found to be similar with that available in Swiss Prot database.

Abstract 103

Predicting Transfusion Requirement by Real Time Hemoglobin Gel Electrophoresis: Improved Diagnostics in Haemoglobinopathy

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Background Transfusion is process of inducting external blood (obtained from blood bank or an individual) in the circulatory system of a patient. A patient with hemoglobin-mediated disorders (e.g. hemoglobinopathy, aplastic anemia, myelodysplastic syndrome or other chronic anemia) may require regular transfusions. The method to evaluate transfusion requirement is mostly empirical in nature and there is no existing methodology (other than direct clinical observation) to objectively quantify the transfusion requirement. *Materials and Methods* A real time interactive multimodal data acquisition imaging setup is developed for the electrophoretic movement of hemoglobin. The electrophoresis of hemoglobin from different hemoglobinopathic patient has been studied. *Result* The electrophoretic modulation by the slow varying periodic pulse cause heme dissociation from hemoglobin (causing hemoglobin band fading), termed as heme knockout, observed for patient blood with varying degree of hemoglobin composition in presence of the periodic pulses. Patients with different degree of transfusion requirement showed different extent of heme knockout effect. *Conclusion* The decision to start transfusions is based on inability to compensate for the low hemoglobin (signs of increased cardiac effort, tachycardia, sweating, poor feeding, and poor growth). The decision making process, based on the aforesaid clinical observation can however be made more effective, given an objective method that would predict the transfusion requirement. We have shown the patient having the unstable hemoglobin content would require transfusion with much higher frequency. Our technique was thus tailor made to distinguish between such differences in hemoglobin stability. Before this method, there was no way by which transfusion rate can be calculated or ascertained for a patient who has been for the first time detected with thalassemia until and unless the signs of anaemia appear.

Abstract 104

p-190 CML Presenting as Extramedullary Blast Crisis: A Case Report

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We present a rare case of Philadelphia chromosome (Ph)-negative chronic myeloid leukemia having p190 BCR/ABL transcript. Patient initially presented with headache and on routine work-up found to have high leukocyte count. His leukocyte alkaline phosphatase score was low but conventional cytogenetics did not reveal any translocation. Reverse transcription polymerase chain reaction detected a minor BCR breakpoint (p-190). P190 BCR-ABL CML represents

only 1% of patients with CML. Morphologically it shows monocytic prominence, it is associated with an inferior outcome to therapy with Tyrosine Kinase Inhibitor(TKI), with few, usually short-lived responses. These patients need to be identified as high-risk patients, monitored closely for efficacy during therapy with TKI, and offered Stem Cell Transplant (SCT) early if eligible for this procedure.

Abstract 105

Response to Danazol in Patients with Aplastic Anemia

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Background The standard therapy of patients with severe aplastic anemia is immunosuppressive therapy with ATG and cyclosporin or allogeneic stem cell transplantation. However, most patients in developing countries cannot afford this form of treatment. Danazol is a synthetic androgen that has been used in patients with aplastic anemia who cannot afford standard treatment. *Objective* We undertook a retrospective study of adult patients with aplastic anemia who were treated with danazol therapy in adult hematology clinic. *Materials and methods* This was a retrospective study in which all adult patients (age >12 years) with diagnosis of idiopathic aplastic anemia treated with Danazol at adult hematology clinic, PGIMER between January 2008 to July 2009 were included. *Inclusion Criteria* (a) Age >12 years. (b) Patients with Idiopathic aplastic anemia diagnosed according to the following parameters: Bone marrow cellularity of less than 25% of normal or less than 50% with haematopoietic cells representing less than 30% of residual cells and, at least two of the following: (1) Neutrophil count less than 0.5×10^9/l, (2) platelet count of less than 20×10^9/l and (3) absolute reticulocyte count <20×10^9/l. (c) Treated with Danazol (starting dose 600 mg/day). The patients were grouped into severe, very severe and non severe aplastic anemia based on following definition: (1) *Severe AA (SAA)*: (A) Bone marrow cellularity <25% or 25–50% with <30% residual hematopoietic cells. (B) Two out of three of the following—(a) Neutrophil <0.5×10^9/l; (b) Platelet <20×10^9/l; (c) Absolute Reticulocyte count <20×10^9/l. (2) *Very severe AA*: As for severe Aplastic anemia but neutrophil <0.2×10^9/l. (3) *Non Severe AA*: Patients not fulfilling criteria for severe and/or very severe aplastic anemia and Neutrophil >0.5×10^9/l. Response to therapy evaluated at 3 months, 6 months, 9 months and at 1 year. Criteria for response to therapy: (a) *Complete Response*: Achievement of all of the following three peripheral blood count criteria: Absolute Neutrophil count >1.5×10^9/l, Hemoglobin >11 g/dl and Platelet count >100×10^9/l, (b) *Partial Response*: Improvement in blood count no longer satisfying the criteria for SAA but not sufficient to meet criteria for complete response with transfusion independence. (c) Overall response includes both complete response and partial response. *Results* All patients of idiopathic aplastic anemia who were registered in hematology clinic PGIMER from January 2008 to July 2009 and were put on danazol therapy were evaluated for response. Out of 25 patients, 5 were very severe aplastic anemia (VSAA), 19 were severe aplastic anemia (SAA) and one patient of non-severe aplastic anemia (NSAA). Danazol was started at dose of 600 mg/day. On follow-up, three patients with SAA and one patient with NSAA had partial response and one patient with SAA had complete response. Four patients died (two of them died because intracranial bleed, one died of febrile neutropeia with sepsis and one patient died of pneumonia), four patients were started on alternative form of treatment (4 and 6 weeks later), 12 patients were lost to follow up. The overall response rate was 20% (complete response + partial response). *Conclusions* The response rates with danazol therapy are 20% among patients with aplastic anemia who cannot afford standard therapy.

Abstract 106

Clinical Profile of NPM1 Positive Acute Myeloid Leukemia from a Single Tertiary Care Center in India

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Molecular markers are increasingly used to risk stratify patients with acute myeloid leukemia (AML). Nucleophosmin gene exon 12 (NPM1) mutation is one such marker which is widely accepted to be associated with a good prognosis especially in the absence of FLT3-ITD mutations. Among patients with standard risk AML the presence of NPM1 mutation is used as a marker to potentially avoid an allogeneic stem cell transplant in first remission. Between 2000 and 2009, retrospective DNA banked sample were available for 460 cases of newly diagnosed AML at our center, either as stored DNA sample or extracted from a cryopreserved cell pellet. The FLT3-ITD and NMP1 mutation were detected using established protocol (Multiplex-Polymerase Chain Reaction amplification of genomic DNA followed by capillary electrophoresis on an ABI 3130 Genetic Analyzer). Of the 460 cases analyzed 112 (24.3%) had the NPM1 mutation. Of these 70 (62.5%) and 26 (23%) were conventional Type A and Type B NPM1 mutations while 16 (14%) were novel NPM1 mutations. The median age of the NPM1 positive cases was 40 years (range 7–71) and there were 55% males. The median WBC count at diagnosis was 31.9×10^9/l (range 1.3–394), the median bone marrow blast percentage was 70% (range 23–98) and the median blast index was 20 (range 0.5–285). Of the myeloid markers CD33 was significantly more positive than CD13 (mean 81.7 ± 22.3 vs. 45.9 ± 33, $P = 0.01$). Sixty of these cases received conventional induction chemotherapy and 39 (65%) achieved CR 1 (3 after 2 cycles). The 2 year KM estimate of OS, EFS and DFS for this cohort was 50.99 ± 8.26, 41.8 ± 8.5 and 75.6 ± 10 respectively. The baseline characteristics of patents with conventional Type A mutation versus those with Type B and novel mutations were comparable with the exception of CD34 positivity on immunophenotyping which was significantly lower in those with Type A mutations ($P = 0.017$). There was no significant difference in the OS, EFS or DFS of patients with the different types of NPM1 mutations. Our findings illustrate that the profile of NPM1 mutations was similar to that reported in the literature with the exception that we could not see a female preponderance with this mutation. The group was associated with an overall favorable outcome in comparison to cases without this mutation. While there are some apparent differences within this cohort based on the type of mutation, from this preliminary analysis this did not appear to impact on the favorable clinical outcome in this group.

Abstract 107

An Effort at Determination of the Reference Range for Platelet Count Amongst the Healthy Population in Eastern India

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Background An indirect platelet count arrived by the counting of specifically labeled platelets relative to the RBCs with a fluorescent flowcytometer (FCM), together with an accurate RBC count determined with an aperture-impedance particle counter has been recommended by ICSH as the reference method for platelet counting. The previous reference method of manual chamber counting of platelets in a phase contrast microscope is laborious with high inter-observer imprecision. Platelet counting in impedance based cell counters (CC) is prone to interference from nonplatelet particulate matter and is known to be inadequate in resolving small RBCs or RBC fragments from normal platelets and normal sized RBCs from large platelets. The occurrence of asymptomatic constitutional macrothrombocytopenia with normal platelet function in a group of blood donors from West Bengal, India, has been reported. While screening college students of West Bengal for thalassemia carrier detection, the mean platelet count and range was observed to be lower than that described in the studies published from the western hemisphere. There are no population-based studies to show the normal range in this region. This study was undertaken as a pilot work to embark upon a future population based study to determine the reference range for normal platelet count for the region and to assess the degree of accuracy of an aperture-impedance particle counter in the normal asymptomatic persons residing in West Bengal for at least 3 years with reference to the flowcytometric platelet counting method. *Materials and Methods* A total of 204 healthy volunteers without any known significant past medical history, including any history of bleeding conditions in either themselves or their relatives were included in the study. All the volunteers were residents of the state of West Bengal, India, for at least 3 years. The age range of the volunteers was 18–46 years. A brief physical examination was performed on all of them. Fresh venous blood to the stipulated amount was collected in 3.0 ml BD Vacutainer® anticoagulated with dipotassium-EDTA. All the specimens were screened for evidence of visual hemolysis and presence of microclots. The specimens were maintained at room temperature (18–22°C), and processed within 4 h of obtaining them. Mixing the specimens was done by at least eight gentle, complete inversions without placing them in any kind of rocking or other "mixing" device. All the specimens were run in a calibrated aperture-impedance particle counter (Sysmex XT-2000i®, Kobe, Japan) within an hour of blood collection and analyzed. A peripheral blood smear was done on all blood samples to examine the red blood cell and platelet morphology. A manual platelet count using improved Neubauer hemocytometer was also done on all specimens. Platelet and RBC enumeration was done in a BD FACSCalibur® FCM. The samples were processed as per ICSH recommendation. Events that were positive for RBC scatter signal as well as for platelet fluorescence were considered RBC-platelet coincidence events and were added to both the RBC and the platelet events to arrive at the R value. The final platelet count was calculated by dividing the RBC count of the specimen obtained from the cell counter by the R value. *Results* Range of platelet count as measured by FCM) was 106.686–338.987 × 10^9/l with a mean of 211.5 (±53.03). The results shown by CC had a range of 64.0–402.0 with a mean of 200.48 (±56.86) × 10^9/l. Considering FCM value as the gold standard it is evident that lower normal values are close to $100 × 10^9$/l and there are 40 participants with values <$150 × 10^9$/l. Comparing the CC results it has been seen that correlation with FCM is 0.749. A result with platelet count >$100 × 10^9$/l in CC were concordant while reports with platelet count <$100 × 10^9$/l were not always so. *Conclusion* Reference range of platelet for the Eastern Indian population may have to be reviewed to bring down the lower normal value to $100 × 10^9$/l and cell counter is a good screening method to detect normal platelet count.

Abstract 108

A Single Centre Experience of Treating CML in A Tertiary Care Centre at Eastern India

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Background Approximately 600 patients are registered at our CML clinic with recordkeeping since 2001 and the longest follow up for 26 years. *Aim of the Study* To document the clinical profile w.r.t. demographic details, treatment received, response to treatment and adverse effects. *Methodology* It is a retrospective analysis of data of 192 CML patients. It includes 118 consecutive patients from Post-Imatinib era and 74 consecutive patients from Pre-Imatinib era to exclude any selection bias. *Results* Age of our patients varies from 4 to 74 years. Median age at presentation is 38 years. One-fifth of our patients presented before 25 years of age. 70% of our patients are male. Commonest presenting symptom was abdominal swelling and abdominal pain, followed by abdominal swelling with fatigue, abdominal swelling with fever, weight loss etc. Commonest presenting sign was Hepatosplenomegaly, seen in 71% of our patients. 5.4% of our patients did not have any organomegaly. Cytogenetic study was done in 94.3% of patients and in 10.4% patients, FISH was performed as there was no dividing cell in cytogenetic study. Additional cytogenetic abnormality was seen in 2.2% patients which included t(3;14), inv4, del14, del2, -3, mar and t(5;22). 97% of our patients presented in chronic phase. 81% of our patients are on Imatinib, with half of them getting Glivec under GIPAP from other centres as our centre is not supported by GIPAP. Complete haematological response(CHR) was achieved in 58.3% of our patients. CHR rate was 70.5% of patients on Imatinib and 21% of patients on Hydroxyurea only. In the pre-Imatinib era 44.6% achieved clinical remission with Hydroxyurea and 39.2% achieved CHR, 86% maintained clinical and haematological remission over 5 years with a median survival of 4.85 years. Commonest side effect of Imatinib was hypopigmentation. Other side effects of Imatinib were seen in 21.6% of patients. 32.3% of these patients had muscle cramp, 11.7% oral ulcers, 11.6% skin rashes, 5.6% GI upset, 5.6% joint pain and 2.9% had pleural effusion. Imatinib was withheld in 22% due to side effects and thrombocytopenia was the cause in one-third of them. One patient conceived and was switched to Hydroxyurea. Cytogenetic response checked in 22 patients, CCyR achieved in 18.4%, MaCyR in 63.7%, MiCyR in 13.3%. Molecular response could be checked only in 5 patients and MMR was achieved in two of them. Imatinib dose was increased in 26.4%. In 44.4% due to suboptimal response, 22.2% due to loss of response, 15.15% due to progression to accelerated or blast phase. Imatinib failure rate was 31.2%. Two patients developed tuberculosis and were started on ATDs. One patient lost response to Imatinib and another progressed to accelerated phase. We did not find any association of outcome with presenting TLC, blast or platelet count. Repeated interruption of Imatinib was associated with worse outcome. Possibly concurrent administration of ATDs was also related to Imatinib failure. *Conclusion* Median age of presentation is one decade earlier in our population with some very young patients. Hepatosplenomegaly was the commonest presenting feature. 70.5% of patients on Imatinib have achieved CHR and even less have achieved complete cytogenetic remission. There was no association with presenting TLC, blast or platelet count with response to Imatinib. However recurrent interruption of Imatinib and coadministration of ATDs was associated with Imatinib Failure.

Abstract 109

Presence of Essential Hypertension and Diabetes Mellitus are Predictors of Intracranial Bleed in Elderly Patients: A Study of 108 Patients of Isolated Thrombocytopenia from a Single Reference Centre

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Introduction Thrombocytopenia poses a significant problem in elderly. It may be a presenting feature of underlying hematologic malignancies which are commoner in this age group. Not only there are varied causes of thrombocytopenia in elderly, it is also associated with significant morbidity and mortality. *Aims and objectives* To study and analyse clinical profile of patients more than 50 years of age with isolated thrombocytopenia. To elucidate causes of isolated thrombocytopenia in elderly patients and to correlate severity of thrombocytopenia and bleeding manifestations with various etiologic factors and co-morbidities which are common in elderly (i.e. diabetes mellitus and essential hypertension). *Methods* One hundred eight patients above the age of 50 year presenting with isolated thrombo-cytopenia (platelet counts <100,000/mm^3 with normal hemoglobin and total leukocyte counts) were enrolled in the study. Detailed history and clinical examination was carried out in each patient. Complete blood counts (CBC) were analysed by automated cell counter. Peripheral smear (PS) was examined in all cases. HbsAg, anti HCV and anti HIV testing by ELISA was done in all patients. Patients were evaluated for hypothyroidism. Wherever clinically indicated bone marrow aspiration and biopsy was done. On clinical suspicion of myelodysplastic syndrome (MDS), bone marrow cytogenetics studies were also done. *Results* Out of 108 patients 102 (94.4%) presented with bleeding as clinical presentation. Twenty-nine (26.8%) presented with serious (WHO grade III/IV) bleeds. According to the etiologies major categories were ITP in 79 (73.1%), MDS in 7 (6.5%), drug induced thrombocytopenia in 7 (6.5%) and connective tissue disorder in 4 (3.7%) cases. Ten patients presented with intra cranial (IC) bleed. On logistic regression analysis comorbidities in form of essential hypertension and diabetes mellitus were significantly associated with occurrence of IC bleed. There was no correlation of serious bleeds with platelet counts. *Conclusions* Isolated thrombocytopenia in elderly is associated with significant morbidity. Diligent clinical and laboratory evaluation is required to elucidate the cause of thrombo-cytopenia in elderly. Comorbidities in this population are associated with more serious bleeds (IC bleed) and not the low platelet counts as is commonly thought of.

Abstract 110

Bone Marrow Metastasis by Solid Tumors-Probable Hematological Indicators and Comparison of Bone Marrow Aspirate, Touch Imprint and Trephine Biopsy

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Background Bone marrow metastasis is an important presentation of solid tumors and early detection of metastasis not only affects the prognosis and treatment but also directly influences the survival of the patients. The present study was conducted to analyze the hematological indicators which can predict the marrow metastasis along with the comparison of bone marrow aspirate, touch imprint and trephine biopsy to define an effective method for its early diagnosis. *Materials and Methods* Retrospective study was undertaken in the Hematology Laboratory of the Institute which included all the cases showing bone marrow metastasis by solid tumors during the time period from January 2006 till August 2009. Thorough bone marrow examination including bone marrow aspirate, imprint cytology and trephine biopsy was done for every case. *Results* The study showed that there was statistical significant difference in mean of Mean Platelet Volume (MPV) and Platelet Distribution Width (PDW) between the cases and controls ($P < 0.001$) and MPV at cut off of <8 fl showed significantly high positive predictive value (100%) and likelihood ratio (21.170) for bone marrow metastasis. Further, bone marrow imprint cytology detected metastatic cells in 96% of cases which was comparable to diagnostic accuracy of trephine biopsy (100%). *Conclusions* Low MPV, an easily available hematological parameter on autoanalyzer, can be used as probable indicator for bone marrow metastasis by solid tumors. In addition, vigilant search of metastatic cells on bone marrow aspirate along with meticulously prepared touch imprint smears is not only as efficient as trephine biopsy for detection of non hematopoietic malignant cells but may also prove an effective method for rapid diagnosis of this grave disease.

Keywords Bone marrow, Solid tumors, Metastasis, Mean platelet volume, Touch imprint

Abstract 111

Understanding Molecular Basis of Protein C Deficiency in Patients with Deep Vein Thrombosis (DVT) from India

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Background Protein C (HPC) is a two chain glycoprotein precursor of vitamin K dependent serine protease, which plays an important role in the regulation of blood coagulation by its ability to inactivate factor V and factor VIII in its activated form. The physiological importance of HPC is evident from the clinical manifestations in individuals with hereditary heterozygote HPC deficiency. *Aims and Objectives* The present study was carried out to provide a molecular basis of protein C deficiency in Indian patients with deep venous thrombosis (DVT) and other disorders which will also provide a basis for antenatal diagnosis for families with hereditary deficiency of Protein C. *Methodology* A total of 118 deep vein thrombosis patients with protein C deficiency were included in the study. Two families of *Purpura fulminans* were also included in the present study. Protein C antigen was estimated by ELISA and the functional protein C was detected by chromogenic assay. Conformation sensitive gel electro-phoresis was used to screen for mutations in these patients which were subsequently confirmed by DNA sequencing. A unique genetic defect named protein C Sapporo mutation which detects type IIb defect was also studied in 300 patients with DVT who had normal levels for protein C, protein S, antithrombin and factor V Leiden. The effect of the three promoter gene polymorphisms of PROC gene on the protein C levels was studied in 98 normal controls and 118 DVT patients. We also studied gamma carboxylase gene and NADPH gene polymorphisms for their possible association with reduced protein C levels. The acquired cause of protein C was ruled out by estimating the descarboxy levels of protein C. Molecular modeling, Western blotting gene expression studies were carried out to explain the defect in certain mutations we detected in our patients. *Results* We detected nine mutations in DVT patients with protein C defect. Three of the

mutations were novel and two were homozygous defect (*Purpura fulminans*). We did not detect protein C Sapporo mutation in our DVT patients. The CG allele polymorphisms in the promoter region of the PROC gene were strongly associated with a reduction of protein C levels in normal population. This could explain the low protein C levels in half the patients where we could not detect any mutation. There was no association between protein C levels and gammacarboxylase gene and NADPH gene polymorphisms. We did not find descarboxy protein C in our patients. Gene expression studies of a novel mutation in the preproregion showed a normal protein C level as compared to the wild type. *Conclusion* (1) Approximately 1/10th of patients with isolated protein C deficiency carry mutations in *PROC* gene; (2) the *PROC* promoter polymorphisms CG were significantly associated with reduced protein C levels in normal population; and (3) sixty percent of our patient population had CG allele and 62% of the patients with this allele had levels between 50 and 70%. As mutations were not identified in the vast majority of the patients, it is possible that the CG polymorphisms in the promoter region of *PROC* gene are the important contributors for low protein C levels in these patients

Abstract 112

Starting Autologous Transplantation in a Non-Hepa Filtered Unit in a Medical College with Severe Resource Constraints

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Objectives To share our experience while starting autologous haematopoietic stem cell transplantation for haematological malignancies in a non-HEPA filtered unit, under severe resource constraints, in a government medical college in Kolkata. *Materials* Both the patients selected for autologous haematopoietic stem cell transplantation (HSCT) were diagnosed cases of multiple myeloma in very good partial response (VGPR). Both of them belonged to the low socioeconomic status, with annual family income less than Rs. 60,000. The transplantations were performed in an air-conditioned room isolated from the general ward, but without a HEPA filter. There was lack of trained nursing staff with previous working experience in a transplantation unit. *Results* The first patient received high dose melphalan at 200 mg/m^2. The total mononuclear cell (MNC) dose infused was calculated as 4.0×10^8/kg and estimated CD34+ cell dose was 2.26×10^6/kg. Patient is doing well at 7 months post-transplantation. The total cost incurred by the patient was Rs. 80,000. The second patient received high dose melphalan chemotherapy. The total mononuclear cell (MNC) dose infused was calculated as 4.0×10^8/kg body weight and the estimated CD34+ cell dose infused was 2.0×10^6/kg body weight. Patient is doing well at 2 months of follow up. The total cost has been Rs. 150,000. Two more transplants have been performed subsequently. *Conclusions*: Our experience while starting autologous haematopoietic stem cell transplantation in non-HEPA filtered rooms and under severe financial constraints has still been extremely encouraging.

Abstract 113

Effect of Imatinib on Platelet Function in Patients with Chronic Myeloid Leukemia

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Background Imatinib, an inhibitor of BCR-ABL tyrosine kinase has become the standard-of-care for upfront therapy in chronic myeloid leukemia (CML). Though Imatinib has revolutionized the management of CML and it is well tolerated by most of the patients, it is not entirely free of adverse effects. A number of well known adverse effects are related to Imatinib, like cytopenia, fluid retention, myalgia, GI disturbances. Sometimes, patients on Imatinib complain of bleeding manifestations, which are mostly related to the degree of thrombocytopenia. However, a minority of patients with normal platelet counts show abnormalities in platelet function, which may or may not be related to bleeding manifestations. *Aim of the Study* In this study, we tried to analyze the effects of Imatinib on platelet function in patients with CML. *Materials and Methods* We studied 28 consecutive CML patients on Imatinib therapy and analyzed their platelet function with collagen, ADP, and epinephrine. The median duration of Imatinib therapy before the test was performed was 154 days. We studied their platelet function by chronolog aggregometer and analyzed the results. Although the lowest cut off of aggregation is different with different reagents and instruments, as a convention, we fixed 60% as the lowest cut off for aggregation with different agonists. *Result* 18 patients out of 28 (64%) showed altered platelet function. This was consistent with previous findings. Interestingly, those who showed lower than 60% aggregation with ADP, also showed lower aggregation with Collagen. Though ADP (10 μM) induced reduced aggregation, it did not show any deaggregation pattern. No patient recruited in this study with defective platelet aggregation demonstrated with ADP had any history of bleeding manifestations. One patient with slightly impaired platelet function showed bleeding manifestations. As ADP and epinephrine are known to be synergistically coupled and so also epinephrine and collagen, there is a theoretical possibility of an indirect dependency of collagen on ADP induced aggregation. In our study, the patients showing lower aggregation with ADP also showed similar results with collagen confirming the possibility of the collagen ADP dependency. The reduced aggregation by ADP was not followed by any further deaggregation phase and hence, although the thrombus formation was reduced, it was a stable thrombus. This may be the probable cause of reduced bleeding manifestations in patients with altered platelet function on Imatinib therapy. *Conclusion*: Imatinib can impair platelet function which might not always manifest clinically. Interestingly occasional patients with imatinib resistance may develop imatinib induced platelet dysfunction. Special concern should be taken to look for imatinib induced platelet dysfunction and whether there is any correlation between imatinib resistance and imatinib induced platelet dysfunction.

Keywords Platelet function, Chronic myeloid leukemia, Imatinib

Abstract 114

Infection and Intracranial Haemorrhage are Leading Causes of Death in Haematology/Haemato-Oncology Patients: Experience from a Tertiary Care Centre

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Introduction Hematology/hemato-oncology patients suffer from high morbidity and mortality due to immunocompromised state,

thrombocytopenia and coagulopathy. This may be prevented by aggressive therapy and better supportive care. *Objective* To assess causes of death in hematology patients with aim to reduce future mortality. *Methods* Retrospectively audit of all patients died in hematology wards from September 2009 to September 2010, with compilation of clinical and laboratory data. *Results* Total no. of deaths during this period: 136. Male: female ratio 2:1, median age 45 years (14 months to 75 years) with 25.7% of patients expired within 24 h of admission. These were patients who presented with septic shock/respiratory failure on ventilator or massive ICH with midline shift. Causes of death are shown in Table 1.

Table 1 Distribution of death in patients with different hematologic diseases

Diagnosis	Number of deaths	Cause of death
Hematologic malignancies	85 (62.5%)	Sepsis (60%), ICH (14%)
Aplastic anemia	43 (31.8%)	Sepsis (51.8%), ICH (40.8%), Cardiac failure (7.4%)
Others	08 (5.7%)	Hemorrhage
Total	136	Sepsis (53%), ICH (24%) Others (23%)

Common underlying diseases were hematological malignancies 62.5% and aplastic anemia 31.8% of patients. Among hematological malignancy, subgroups were AML (36.1%), B-ALL (20.2%), CML-BC (10.5%), APML (7%), T-ALL (5.8%). Others include ITP, Factor X deficiency, and thalassaemia. *Conclusion* This highlights the poor prognosis in patients with pre existing infections or large ICH in the group of hematology in-patients. Early referral and diagnosis is needed to prevent these deaths.

Abstract 115

Cytogenetic Profile of Acute leukemia: Single Centre Study from India

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Background Cytogenetic studies of acute leukaemia cases have shown that most of the patients have non random clonal cytogenetic abnormalities. Further molecular studies of these abnormalities have revealed specific genetic abnormalities involved in the leukemogenesis. Gene activation and fusion as a result of chromosomal translocation or inversion have been implicated in the disordered proliferation and differentiation of the leukaemic clone. Cytogenetics has now emerged as an important tool for the diagnosis, classification, prognostication, risk stratification and as a guide to the clinicians for providing appropriate therapy. However unfortunately there is scarcity of data on cytogenetic profile among acute leukaemia patients in India. *Aims* (1) To determine the incidence of abnormal karyotypes among the acute leukaemia patients in our centre; (2) to determine the more common cytogenetic abnormalities among AML (acute myeloid leukemia) and ALL (acute lymphoblastic leukemia) patients. *Methodology* All the cases were examined clinically and those who had features of acute leukaemia like pallor, generalized lymphadenopathy, hepatosplenomegaly, sternal tenderness, bleeding manifestations and fever were advised to undergo bone marrow aspiration and biopsy. Light microscopy, cytochemistry was done on leukaemia cases and samples were further sent for immunophenotyping and cytogenetics. Cytogenetic study was done following unstimulated cell culture of bone marrow samples and GTG banding of all the 33 diagnosed cases of acute leukaemia. *Results* Among the ALL (20) cases, 15 were <18 years of age and 5 patients were ≥18 years. Normal karyotype was observed in 6/20 cases i.e. 30% cases. Hyperdiploidy which is a good prognostic indicator was the commonest cytogenetic abnormality observed in 4/20 i.e. 20% of cases followed by t(4;11) and Ph chromosome positive cases both of which are bad prognostic indicators, observed in 3/20 i.e. 15% cases each. Out of the three Ph chromosomes positive cases two were adult (age ≥18 years) patients. Among the AML (13) cases four patients were <18 years of age and nine patients were ≥18 years. Five patients (38%) had normal karyotype, all of whom are adults, while 62% were having some cytogenetic abnormality. Trisomy 8 and t(15; 17) were the commonest abnormalities among the AML cases both of which are observed in 3/13 i.e. 23% of cases. Del 11q23 was the only cytogenetic abnormality observed in both AML and ALL cases. *Conclusion* (1) Cytogenetic abnormalities were present in majority (62%) of AML cases and (70%) of ALL cases; (2) hyperdiploidy was the commonest cytogenetic abnormality among the ALL patients followed by t(4;11) and Ph positive cases; (3) trisomy 8 and t(15;17) were the commonest cytogenetic abnormalities among AML cases; (4) cytogenetic abnormalities were more common in childhood AML as compared to adult AML and (5) Del 11q23 is the only abnormality observed in both AML and ALL cases.

Abstract 116

Prevalence of Glanzmann's Thrombasthenia in of West Bengal, India

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Background Glanzmann thrombasthenia (GT) is a rare, autosomal recessive bleeding disorder characterized by a life-long mucocutaneous bleeding tendency, absent or severely reduced platelet aggregation in response to the physiological agonists adenosine 5′-diphosphate (ADP), epinephrine, and collagen, and a relatively normal initial phase of aggregation in response to ristocetin. The disease is caused either by a lack or dysfunction of the platelet integrin $_{IIb}\beta_3$ (glycoprotein IIb/IIIa), which serves as a receptor for fibrinogen, von Willebrand factor, and perhaps other adhesive glycoproteins. This study reveals the occurrence of GT among the patients attended Hematology OPD and perhaps it is the 1st report on the prevalence of GT from West Bengal. *Aims* This study aims to evaluate the prevalence of Glanzmann's thrombasthenia (GT) and their clinical profile amongst patients presented with bleeding manifestations. *Materials and Methods* Study population included patients attending Hematology OPD with bleeding manifestations from November 2009 to July 2010. A detailed history was taken and thorough physical examination was done in each case. A complete hemogram was done and peripheral blood smear was examined for platelet count and morphology. A coagulation screening (PT/APTT &TT) was performed. The patients with normal platelet count and normal coagulation parameters underwent platelet function tests and clot retraction test.

Platelet function study was done using agonists Ristocetine (1.25 mg/ml), ADP (10 μM), ADR (10 μM), Collagen (2 μg/ml) and Arachidonic acid (0.5 mM) in Lumi-aggregometer and result was recorded on chart paper as the percentage of aggregation versus time of aggregation. *Result* The study was conducted on 135 patients aged 3–70 years who presented with muco-cutaneous bleeding manifestations and 26 healthy control subjects. Out of these 6 (4.44%) patients were diagnosed as GT depending on the basis of above parameters. Mean age of patient diagnosed with GT 26; male:female ratio among patients is 1:2 and no consanguinity has been reported. Four (66.6%) patients had a positive family history. One (16.6%) of six patients was diagnosed as acquired GT. Most common bleeding manifestation was gum bleeding and presented by 66.7% of GT patients followed by menorrhagia (50%), epitaxis and bleeding P/R. In all cases the aggregation with all agonists expect Ristocetine found to be absent and normal with Ristocetine (50–70%) in five cases. In one case, the aggregation response with Ristocetine was reduced (31.25%). *Conclusion* This study demonstrates that GT accounts for a significant percentage among all other the platelet function disorders in the patients of West Bengal and perhaps, gum bleeding is the most common clinical representation of the disease in this state.

Abstract 117

Diagnosis of PNH by Flowcytometry

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Background Flowcytometry acts as a rapid, sensitive and reproducible diagnostic tool for the detection of PNH clones in different PB cell populations and has now become the 'Gold Standard' for diagnosis of PNH. Although flowcytometry for diagnosis of PNH is being used over two decades; there are no set guidelines available for sample processing and selection of reagents. Hence standardizing these tests in a clinical laboratory remains challenging. *Methods* Different staining protocols (stain-lyse-wash-method, Lyse- stain-wash-method, No-lyse no-wash method) use of FLAER with different GPI markers (CD55, CD16, CD66b, CD24, CD59) gating strategies (SSC/CD45, SSC/CD15, and SSC/CD33) were compared for neutrophil staining. Similarly for RBC, the effects of different dilutions and additional washing steps were compared. *Result* Among different methods for neutrophils staining, stain-lyse-wash method gave optimal staining for all the GPI markers studied. In this CD55, CD16, CD66b, CD24 and FLAER gave better results as compared to CD59. FLAER as a single agent in a simple and fast whole blood staining procedure was shown to be equally effective as compared to conventional immunophenotyping. FLAER was more sensitive than single parameter (CD55 or CD59) analysis on neutrophils due to its high signal to noise ratio and gave better separation of Type I, II and III cells. Combining FLAER with multiparametric flowcytometry can further improve the sensitivity and specificity of the test. For RBC, 1:150 dilution of sample with two additional washing steps after staining enhanced separation of different types of PNH cells and CD59 was found to be better than CD55.

Abstract 118

Immunophenotypic Profile Newly Diagnosed and Relapsed Patients with Acute Promyelocytic Leukemia and its Impact on Clinical Outcomes

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Acute promyelocytic leukaemia (APL) has a unique immunophenotypic (IPT) profile at diagnosis. There is limited data on the profile of relapsed APL. There is some data to suggest that the IPT profile has an impact on clinical outcomes such as bleeding, differentiation syndrome (RAS) and relapse (Lo Coco et al. Leukemia 2007). We undertook a retrospective study to look at the IPT profile of newly diagnosed and relapsed cases with APL diagnosed at our center. Between January 2006 and August, 128 newly diagnosed cases and 18 relapsed cases were seen at our center. A complete IPT profile was available on 100 newly diagnosed cases and 13 relapsed cases and these were evaluated for this analysis. Bone marrow samples from newly diagnosed and relapsed cases were utilized for these studies. IPT was done using a panel of monoclonal antibodies to CD13, CD33, CD34, CD19, CD38, CD49d, CD49e, CD184 and HLADR directly conjugated with FITC, PE, PerCP or APC and analyzed using a FACSCalibur and CellQuest-Pro software (IPT was done in our laboratory as part of the routine diagnostic procedure for leukemia). To enhance objectivity of the analysis median fluorescence intensity (MFI) of the markers analyzed was used preferentially. The median age of the newly diagnosed and relapsed cases was 29 years (range: 4–60 years) and 21 years (range: 7–55 years). The median WBC count of relapsed cases was significantly lower than in newly diagnosed cases ($P = 0.01$). There was no significant difference between these two groups for any other baseline clinical, laboratory or molecular markers (bcr isoforms). Morphologically there was no significant difference between newly diagnosed and relapsed cases. Table 1 summarizes MFI of the common IPT markers between these two groups. As shown in Table 1 relapsed patients with APL had a significantly higher expression of CD34 and a significantly lower expression of CD13 and CD38. Of the 100 newly diagnosed patients evaluated in this study 82 received an ATO based therapy while 18 received an ATRA/chemotherapy based regimen. All relapsed patients received both ATO and ATRA as part of induction therapy. Of all patients studied 29 developed features of a RAS. With the exception of a low CXCR4 level (median MFI 46 vs. 57.2; $P = 0.008$) none of the IPT markers correlated with the development of RAS. Similarly there was a correlation with low VLA-5 expression and the development of IC bleed (median MFI 362 vs. 542; $P = 0.047$). The 1 year Kaplan–Meier estimate of OS and EFS of newly diagnosed and relapsed cases of APL was 77.22 ± 4.3 vs. 48.61 ± 14.8 and 74.7 ± 4.5 vs. 48.61 ± 14.8 respectively. There was no correlation in this analysis between expression of CD34, CD13 or the adhesive molecules evaluated and OS or EFS. However, there was a negative correlation between the MFI expression of CD33 with OS and EFS ($P = 0.024$ and 0.021). On a uni-variate analysis additional the WBC, Platelet and serum creatinine values at diagnosis along with relapsed APL had a significant impact on EFS. On a multi-variate analysis after adjusting for these parameters the MFI of CD33 retained its protective effect while WBC count and relapsed APL

retained their negative impact on EFS. In conclusion there are significant IPT differences between newly diagnosed and relapsed cases of APL. There are correlations with some IPT markers and complications unique to this subset of leukemia. Additionally IPT markers could potentially serve as prognosticators of clinical outcome though this remains to be validated in a larger study with longer follow up.

Table 1 Immunophenotypic markers of newly diagnosed and relapsed cases of acute promyelocytic leukemia

Marker	MFI values		P value
	New $n = 100$ Median $\pm$ SD	Relapse $n = 13$ Median $\pm$ SD	
CD 13	361.9 $\pm$ 514.2	171 $\pm$ 340.4	0.009
CD 33	397.7 $\pm$ 291.2	491 $\pm$ 390.7	0.746
CD 34	34.4 $\pm$ 114.2	65.5 $\pm$ 174.9	0.037
CD 38	102.8 $\pm$ 82.3	37.4 $\pm$ 27.7	0.000
HLA DR	33.6 $\pm$ 116.7	28.9 $\pm$ 26.9	0.325
VLA 4	295.5 $\pm$ 218.4	201.6 $\pm$ 164.6	0.135
VLA 5	513.9 $\pm$ 300.8	333.7 $\pm$ 283.4	0.166
CXCR 4	50.9 $\pm$ 53.3	67.3 $\pm$ 45.9	0.650

Abstract 119

Expression of Ara-C Metabolizing Enzymes Mediates In vitro Sensitivity to Ara-C in Primary AML Cells from Patients with de novo AML

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Background Wide inter-individual variation in terms of treatment outcome and toxic side effects of treatment exist among patients with AML receiving chemotherapy with cytarabine (Ara-C) and daunorubicin. The pre-requisite for the cytotoxic action of pro-drug Ara-C is the enzymatic conversion to its active tri-phosphorylated form Ara-CTP. Many drug activating (Deoxycytidine kinase (dCK) and human Equilibrative Nucleoside Transporter 1 (hENT1) and deactivating (Cytidine deaminase (CDA), 5′nucleotidase (NT5C2) and ribonucleoside reductase (RRM1) genes which are involved in metabolism and biotransformation of cytarabine contribute to the variation in Ara-C sensitivity in AML patients. The objectives of the present study were: (i) to analyze the extent of variation in Ara-C metabolizing genes' expression in AML patients and normal controls and (ii) to determine the role of mRNA expression of Ara-C metabolizing genes' on in vitro Ara-C sensitivity. *Methodology* Bone marrow sample from 74 adult patients with de novo AML (other than AML-M3), as well as peripheral blood samples from thirty six normal healthy volunteers were included in this study. Total cellular RNA was extracted using Tri Reagent and cDNA was synthesized. mRNA expression levels for each target gene relative to housekeeping gene GAPDH was analyzed using Taqman based gene expression assays. In vitro cytotoxicity was assessed using MTT cell viability assay and IC-50 was calculated. Patients were classified as sensitive and resistant based on the IC-50 values <5 μM and >5μM respectively. *Results and Conclusions* There was 35–2,500 fold variation in mRNA expression in candidate

Ara-C metabolizing genes in AML patients and 20–30 fold variation in normal controls (Fig. 1). The mRNA expression levels of dCK, hENT1 and RRM1 were significantly higher and CDA and NT5C2 significantly lower in AML patients compared to normal controls (Fig. 1). When Ara-C IC-50 values were compared with the mRNA expression levels of these candidate genes, Ara-C sensitive patients ($n = 17$; IC-50 <5 μM) showed significantly higher mRNA expression of hENT1 and dCK compared to those with Ara-C resistant ($n = 37$) IC-50 >5 μM (median 145 (59.67–657.6) vs. 86 (37.49–322.1), $P = 0.0015$ and median 298 (61.46–1232) vs. 154 (31.87–749.2)), $P = 0.01$ respectively). In conclusion, we have shown that RNA expression of dCK and hENT1 mediates invitro sensitivity to Ara-C and might be involved in resistance to Ara-C in AML patients. The expressional status of these enzymes could be predictors of treatment outcome as well as for optimizing the therapy.
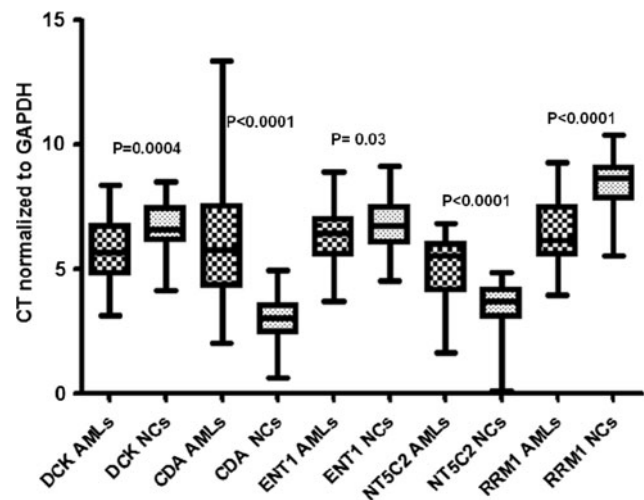

Fig. 1 mRNA expression of Ara-C metabolizing genes expression in AML patients and normal controls

Abstract 120

Methylation of p15INK4B and its Association with Deletion of Genes on Chromosome Arm 7q: First Report from India

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Background Myelodysplastic Syndromes (MDS) are clonal hematological disorders that frequently represent an intermediate disease stage before progression to acute myeloid leukemia (AML). Although several cytogenetic abnormalities have been associated with this heterogenous disorder, little is known about what genetic changes are responsible for the disease. Recent studies have elucidated that not only genetic alterations but also epigenetic changes may play an important role in carcinogenesis. It is reported that MDS patients from India have severe clinical presentation and their response to treatment is also poor as compared to the west. Very limited data is available on methylation studies from India. In view of this, we evaluated the methylation status of p15 in a series of 30 MDS patients. *Aim of the Study* To investigate the frequency of p15 gene methylation in MDS

patients and its correlation with cytogenetic abnormalities. *Methodology* Total genomic DNA was extracted from bone marrow/peripheral blood leucocytes of MDS Patients. Bisulphite treatment of DNA was performed. Bisulfite modified DNA was amplified by methylation specific PCR. Cytogenetic analysis was performed using GTG banding and karyotyped according to the International System for Human Cytogenetic Nomenclature (ISCN). *Results* A total of 30 patients (median age 40 years, range 14–75 years; M:F 3:2; median TLC $4.15 \times 10^9/l$, range $0.95–116 \times 10^9/l$; median platelet count $102 \times 10^9/l$, range $5–274 \times 10^9/l$; median hemoglobin 6.7 g/dl, range 2.9–16.1 g/dl, were studied. Aberrant methylation of p15 was detected in 12/30 (40%) patients. Chromosomal study results were available for 24 patients. Twelve patients (50%) showed chromosomal anomalies. The frequency of normal karyotype (25%) was lower in patients with p15 methylation as compared to patients with unmethylated p15 (60%). Monosomy 7 was detected in 4 patients and p15 was methylated in two of these cases (50%). The frequency of abnormal karyotype in different WHO subtypes was as follows: 75% in RCMD and RAEB-2, 67% in RAEB-1, 33% in RARS, and 20% in RA. *Conclusion* P15(INK4B) gene methylation is a frequent event in patients with Myelodysplastic Syndromes and has correlation with the karyotypic pattern.

Abstract 121

Gastrointestinal Lymphomas: The Pattern of Distribution and Clinicopathological Profile of Unusual Cases in a Tertiary Care Centre: A 10 Year Experience

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Background One of the major sites of extra nodal lymphomas is the gastrointestinal tract. The involvement of gastrointestinal tract by lymphomas can be primary or secondary. Primary gastrointestinal lymphomas are almost always non-Hodgkin lymphomas (NHL). These represent 10–15% of all NHL cases and about 30–40% of extra-nodal lymphomas. They show an apparently increasing incidence trend worldwide. In Western countries, the most commonly affected site is the stomach followed by the small bowel, ileum, caecum, colon and rectum. Considerable variation exists in the literature with respect to incidence of the various histological subtypes and sites of involvement. *Aim* This study was undertaken to ascertain the anatomic distribution and histological subtypes and sites of all gastrointestinal lymphomas in southern India since there is no large study documented in the literature. *Methodology* 366 patients over a period of 10 years (2001–2010), with histopathological diagnosis of lymphoma involving the gastrointestinal tract (both primary and secondary) were analyzed retrospectively. These included 319 mucosal biopsies and 47 resected specimens. All lymphomas were reclassified according to the WHO 2008 classification. *Results* These 366 cases consisted of 297 males (81%) and 69 females (19%) with a male: female ratio of 4.29:1. The mean age was 45 years (range 3–88). DLBCL was the commonest subtype (245 cases, 66.94%) followed by low grade MALT lymphoma (39 cases, 10.66%) and Burkitt's lymphoma (34 cases, 9.29%). The common sites were stomach (183 cases; 50.52%), small intestine (106 cases; 28.96%) and colon (72 cases; 19.67%). Bone marrow involvement was seen in 14/259 (4.5%) cases. The unusual gastrointestinal lymphomas documented during the study are listed in the table below

Diagnosis	Number of cases
NHL with concomitant adenocarcinoma	4
Composite lymphoma (low grade MALT with concomitant Hodgkin lymphoma)	1
Burkitt's lymphoma with renal cell carcinoma	1
Plasmablastic lymphoma	6
Post transplant lymphoproliferative disorders	8
T-cell NHLs including	
NK/T cell lymphoma 2	16
Enteropathy associated T-cell lymphoma 1	
Anaplastic large cell lymphoma 5	
Peripheral T-cell lymphoma 8	
IPSID	5
Hodgkin lymphoma	2
CLL/PLL	1

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Adolescent and Young Adult Lymphoma: Single Center Experience

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Introduction Management of Non-Hodgkin lymphomas (NHL) in adolescent and young adult (AYA) has remained a topic of debate over the years. These patients are treated either on pediatric or adult protocols based on the treatment centres' expertise. We present the epidemiology and treatment outcome results of AYA aggressive lymphomas treated at a tertiary care centre using both adult and pediatric protocols. *Materials and Methods* AYA lymphoma patients (16–25 years) with confirmed diagnosis of NHL registered in lymphoma clinic from January 2007 to May 2010 were included in this retrospective analysis. Data on the baseline clinical characteristics, histological subtypes, stage, and laboratory values was recorded for epidemiology analysis. Patients were managed with pediatric or adult protocols as per the lymphoma clinic decision. Response to therapy and follow up was done as per the standard of care. *Results* 2,745 patients of newly diagnosed NHL were registered in lymphoma clinic. Of these 122 (4.4%) patients were of AYA NHL (103 males and 19 females). The frequency of various histological subtypes: DLBCL 50, Burkitt's lymphoma 13, Burkitt's like DLBCL 12, T-lymphoblastic lymphoma 21, ALK-negative ALCL 7, ALK-positive ALCL 9 and other subtypes 7. Fifty percent of patients had stage III/IV disease. Seventy-nine patients had increased LDH. Adverse features which can potentially affect the treatment intensity like anemia and hypoalbuminemia at presentation were seen in 31 and 35 patients respectively. Seventy-two patients were treated. Forty-one patients received chemotherapy with pediatric protocols (BFM 90, MCP 842, MCP 841) and 31 patients were treated on adult protocol (CHOP or CHOP like chemotherapy). Response evaluation data at the end of planned chemotherapy was available in 66 patients. Complete remission was achieved in 47 patients, while partial responses and progressive disease was seen in 4 and 15 patients respectively. At

median follow up of 14 months (range from 1 to 43 months), 42 patients remained in CR while relapse and progression was seen in 3 and 4 patients respectively. Death due to disease progression occurred in 15 patients. 25 patients developed febrile neutropenia with median duration of hospitalization for 12 days [range from 3–45 days]. Treatment related death occurred in 3 patients. At median follow up of 14 months, out of 20 DLBCL patients 16 (80%) are in clinical remission with equivalent results with either adult or pediatric protocols. *Conclusion* A significant proportion of AYA NHL presents with adverse features. DLBCL followed by T lymphoblastic lymphoma is the main histological subtype in this subgroup at our centre. Aggressive pediatric protocols were well tolerated in adolescent patients. Comparable outcomes can be achieved in adolescent DLBCL patients with either adult or pediatric protocols.

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Two Unusual Presentations of Follicular Lymphoma

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Follicular lymphoma (FL) is the second most common subtype of Non Hodgkin's Lymphoma (NHL). Patients with FL often present with painless waxing and waning peripheral adenopathy. We describe two cases with rare clinical manifestations requiring admission. The first case was a 48 year old male who presented with fever, left sided chest pain, abdominal distension and neck nodes for 1 month. Initial evaluation revealed generalised lymphadenopathy with organomegaly. Investigations suggested a chronic lymphoproliferative disorder, non-CLL, non-HCL type. He progressively worsened 3 weeks later with increasing dyspnea and abdominal distension. A repeat lymph node biopsy was done as the initial one was inadequate and was reported as Follicular Lymphoma grade 1. He required urgent laparotomy for spontaneous splenic rupture which was suggested by examination and imaging when his haemoglobin % dropped. The next case was a 66 year old diabetic male who was admitted for evaluation of intermittent fever with significant weight loss, back pain for 6 months and anemia requiring repeated transfusion for 2 months. Examination revealed no organomegaly or lymphadenopathy. The initial evaluation revealed normocytic normochromic anemia with no abnormal cells on the smear. He had no lytic lesions on the skeletal survey and no M band. He developed weakness in his right leg on day 2 of his admission which progressively worsened to paraplegia on day 4. MRI revealed extradural compression in the cervico-dorsal region. The bone marrow aspirate was dry, the biopsy was consistent with NHL. He underwent urgent resection of the extradural mass with laminectomy. The tissue biopsy was reported as grade 1 Follicular Lymphoma. Although these manifestations occur usually in a high grade lymphoma, an indolent lymphoma must be kept in mind during the work up.

Abstract 124

Immunosuppresive Therapy with Antilymphocyte (ALG)/ Antithymocyte Globulin (ATG) for Aplastic Anemia: A Single Centre Experience

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Background Immunosuppressive therapy (ATG/ALG) is treatment of choice for patient with aplastic anemia without a histocompatible sibling. There is limited data available on the efficacy of ATG in Indian patients. *Aim* To analyze the response to immunosuppressive therapy with ATG/ALG in adult patients with aplastic anemia treated between 1985 and 2009. *Methodology* This is a retrospective analysis of data of patients who received treatment with either ALG or ATG between 1985 and 2009. Initially, between 1985 and 1994, patients received ALG alone while from 1995, all patients were planned to receive cyclosporine for 1 year following treatment with ALG or ATG. Response was confirmed based on EBMTR guidelines for management of aplastic anemia. *Results* Two hundred and eighty-two patients with a median age of 45 years (range 15–83) were studied including 196 (69.5%) males and 86(31.5%) females. Forty-four patients (15.6%) had very severe aplastic anemia (VSAA), 149(52.8%) had severe aplastic anemia (SAA) and 89 (31.6%) had non severe aplastic anemia (NSAA). An objective response to treatment was seen in 179 (63%) which included a complete response (CR) in 76 (27%) and partial response in 103 (36%) patients. In VSAA, SAA, NSAA the objective response (CR + PR) achieved was as follows 56.8% (25 of 44 patients), 61.7% (92 of 149 patients), 69.7% (62 of 87 patients) respectively. The objective response achieved in ALG, ALG + CSA, ATG + CSA group is as follows 52% (52 of 100 patients), 66.7% (64 of 96 patients) and 73.3% (63 of 86 patients) respectively. Thirty-two (11.34%) patients died due to infection in the first month following treatment with ATG/ALG. Hundred patients (35.46%) suffered from serum sickness following treatment with ATG/ALG. On follow up, 12 patients (4.2%) lost response while 10 (3.5%) underwent clonal evolution into paroxysomal nocturnal haemoglobinuria, myelodysplastic syndrome or acute myeloid leukemia). Five (1.7%) patients who did not show response/had a clonal evolution underwent allogeneic bone marrow transplantation. The 5 year overall survival (OS) for the entire cohort is 64%. Factors predicting a good response to ALG/ATG included the diagnosis of non severe aplastic anemia and the addition of cyclosporine to ATG/ALG. *Conclusion* Immunosuppression with ATG/ALG induces a response in 63% with a 5 year OS of 64%. Combining ATG/ALG with cyclosporine has helped in improving results. These results suggest that this is a viable option for adult Indian patients who cannot have a bone marrow transplant for aplastic anemia.

Abstract 125

Outcome After Hematopoietic Stem Cell Transplantation in Children with Hemophagocytic Lymphohistiocytosis at the Hospital for Sick Children, Toronto (SickKids)

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Background Hematopoietic stem cell transplantation (HSCT) is the only curative option for patients with primary hemophagocytic-lymphohistiocytosis (HLH) as well as for patients with secondary HLH who fail to respond to therapy. *Objectives* To report outcomes and HSCT complication of pediatric patients with HLH. *Methods* A retrospective chart review of pediatric recipients of HSCT for HLH between June 1995 and Aug 2009 at SickKids Hospital. Event free survival was defined as no evidence of relapse, graft failure (GF) or death. Univariate analysis was utilized to assess factors influencing survival. *Results* Eighteen children (10 male), median age 1.2 years (range 5 months to 16 years) received a HSCT for HLH. Fourteen children had primary HLH. At the time of transplant, 2 children had active HLH and 2 were in partial remission. Median time to HSCT from diagnosis was 6 months (range: 3–39 m). Donor source was; sibling marrows 6, cord blood stem cells 6, living unrelated stem cells 5 and haplo-transplant 1. Thirteen children received fully matched donor stem-cells. Sixteen received myeloablative and 2 received

reduced intensity conditioning. Median time to neutrophil engraftment in 14 children was 16.5 days (12–29 days). Three children had primary GF and 1 died before D + 28. Thirteen children had bacterial and/or viral infections and 7 had veno-occlusive disease of the liver. Nine children required intensive care unit admission. Eight children had acute and three developed chronic GVHD. Overall actuarial survival at 3 years was 60%. Three patients died from multi-organ failure before day +100, and another patient died from pulmonary hemorrhage after day 100. Two children with primary GF developed recurrent HLH and died from complications after a second HSCT. One patient developed anaplastic large cell lymphoma. Three of 4 children not in complete remission at the time of transplantation died. Eleven children (61%) are alive at a median follow-up of 55.5 m (4.5–122 m). The following factors were not statistically associated with survival: acute GVHD, stem cell source, underlying disease or conditioning regimen. *Conclusion* Early mortality prior to day 100 was low (16%). Overall survival of 61% is similar to most published series. High infection rate and organ dysfunction complicate outcome. Reduce intensity conditioning may improve outcome after HSCT in HLH.

Abstract 126

Iron Status in Children with Behavioural Disorders

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Introduction Iron deficiency anemia (IDA) is a major public health problem in India. Iron plays an important role in cognitive function and behavioural development. It is an essential element involved in myelin formation, neurometabolism and neurotransmitter synthesis. Several behavioral disturbances have thus been reported in children with IDA. Despite the frequency of IDA in India, a search in literature revealed paucity of information on iron status and behavioural disorders. *Aim* To assess iron status in children with behavioural disorders and improvement in hematological profile, iron status and behaviour after iron therapy. *Materials and Methods* This was a prospective study done on 44 children (age 3–12 years) with behavioural disorders as per DSM IV criteria. Thirty age-matched controls were also included. Child behavior check list (CBCL) was applied on all patients. Complete blood counts and iron profile [serum iron, TIBC, % transferrin saturation (TS), and serum ferritin] were estimated in all cases. Children found to be iron deficient (TS $< 16\%$, and/or SF < 16 µg/l) were given iron therapy for 100 ± 10 days and were reevaluated for hematological and clinical improvement at the end of treatment. *Results* Iron deficiency was found in 32 (73%) children. Of these 32 patients, iron deficiency was latent in 4 (12.5%) patients. There was a statistically significant ($P < 0.001$) improvement in hematological parameters, iron status, and clinical features as assessed by CBCL scores. *Conclusion* The presence of iron deficiency in children with behavioural disorders and subsequent improvement in clinical features and iron status with iron therapy suggests a possible causal relationship between iron deficiency and behavioural disorders. Larger studies with longer periods of follow up are required to further substantiate these findings.

Abstract 127

Incidence of Immunodeficiency Virus, Hepatitis B Virus & Hepatitis C Virus and Syphilis in Donor's Blood

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Objectives Voluntary donor selection and screening of donor's blood for infective agents are the cornerstones of transfusion medicine. Strict donor selection criterion, proper counseling and deferred collection may reduce wastage of resources. *Materials and Methods* During the period of 01.01.2007 to 31.12.2008, a total number of 44,183 units of blood were collected from healthy voluntary donors. There were 39,744 males and 4,439 females with a male: female ratio of 9:1. Age ranged from 19 to 59 years. Blood was collected in CPDA-1 bags. *Discussion* All blood samples were tested for HIV1 and HIV2, Hepatitis B surface antigen, Hepatitis C and VDRL (REAGIN) for syphilis. It was observed that 283 tested positive for HIV (0.64%), 1,001 were positive for HbsAg (2.3%), 717 were positive for HCV (1.6%) and 577 (1.3%) were Reagin (VDRL) positive. Total 2,578 units (5.8%) of blood was discarded due to presence of infective agents. *Conclusion* Strict quality control, proper counseling of donors and training of blood transfusion personnel including deferring of suspected donors may help wastage of huge resources and reduce inventory.

Keywords Human immunodeficiency virus, Hepatitis B surface antigen, Hepatitis C, Reagin, VDRL, Voluntary donors, CPDA-1

Abstract 128

Burden of Iron Overload

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Background 46 years Hindu male, known HbE-beta thalassemia patient, presented on 31st May 2010 with c/o severe low back pain associated with tenderness over ribs and both arms along with easy fatiguability and exertional breathlessness for last 6 months, which was aggravated for last 2 weeks. *Aim of Study* To detect iron overload and its complications in a thalassemia Intermedia patient having 10 units blood transfusion in his lifetime. *Methodology* History taking, clinical examination, radiological examination, echocardiography and follow-up. *Results* The patient was found to have HbE-beta thalassemia and hypersplenism, together with endocrinopathy, osteoporosis, cardiomyopathy, chronic liver disease related to hemosiderosis. *Conclusion* Iron overload can occur without many units of blood transfusion in thalassemia Intermedia patients.

Abstract 129

Frequency of *BCR-ABL* Fusion Transcripts in Patients with Chronic Myeloid Leukemia

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Background Presence of translocation (9;22) resulting in a fusion transcript *BCR-ABL* is a hall mark of chronic myeloid leukemia (CML). The most common types of *BCR-ABL* fusion transcripts in CML are b3a2 or b2a2 type resulting in BCR-ABL p210. In addition, P190 (e1a2 type fusion) as well as relatively rare variant fusion transcripts (b3a3, b2a3, e19a2) may also occur. *Aim of the Study* The present study was aimed at determining the frequency of *BCR-ABL* fusion transcripts in CML patients that were analyzed by reverse transcriptase polymerase chain reaction (RT-PCR). *Methodology*

Between Jan 2000 till 2010 August, a total 583 patients with CML were tested for the presence of *BCR-ABL* fusion transcript by RT-PCR at our institution. RNA was extracted from peripheral blood samples, cDNA was synthesized using Superscript first strand cDNA synthesis system and PCR was done to check for the presence of major (b2a2 or b3a2-p210), minor (e1a2-p190) as well as micro (e19a2-p230) types fusion transcripts. The presence of rare variants (e19a2 and b3a3) was confirmed by sequencing of the PCR products. *Results* There were 361 patients with b3a2 (62%), 201 patients with b2a2 (34%), 10 patients with e1a2 (1.7%), 3 with e19a2 (0.5%) and 1 with b3a3 (0.2%) fusion transcripts. In 8/10 e1a2 positive patients, e1a2 was the sole abnormality while in the remaining 2, this co-existed with b2a2 fusion transcript. Only few previous reports are available on Imatinib treatment in CML patients with non-major BCR-ABL transcript variants. The e1a2 transcripts have been shown to coexist with b2a2/b3a2 transcripts, but their presence as the only transcript in CML is very rare. The largest study till date from MD Anderson in 1292 CML patients revealed e1a2 fusion transcript frequency of 1%. The present study is the second largest study in CML reporting e1a2 positive *BCR-ABL* in CML. Recent reports have shown that CML with e1a2 BCR-ABL fusion transcripts is associated with monocytosis and an inferior outcome to therapy with tyrosine kinase inhibitor (TKI), with responses being usually short-lived. These patients need to be identified as high-risk patients and monitored closely for efficacy during therapy with TKI. The e19a2 fusion transcript that was initially suggested to be associated with neutrophilic CML, a milder form of BCR-ABL positive myeloproliferative disease, has also been rarely reported in classical CML. The b3a3 *BCR-ABL* transcript is exceptionally rare; only eight such cases have been reported in CML till date, the prognosis of patients with this transcript is controversial. *Conclusion* In conclusion, identifying the type of *BCR-ABL* fusion transcript, especially the rare variants in CML, may have clinical relevance in understanding the prognosis of treatment.

Abstract 130

Rare Combination of Codon15(−T) Mutation of *β* Globin Gene and HPFH Resulting in Thalassemia Intermedia Phenotype: A Case Report

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Beta thalassemia intermedia phenotype results from various genotypic combinations which include combinations of the mutations in the *β* globin gene and Co-existence of *β* globin gene mutations and HPFH. Here by we report a case with a rare combination of HPFH and *β* thalassaemia which resulted in thalassaemia intermedia phenotype. A 3 year old male child presented with anaemia requiring blood transfusions. On investigations he had low Hb with abnormal RBC indices and a Hb F of 100% (by HPLC). The father had normal RBC indices and elevated Hb F of 35%. The Mother showed heterozygous for beta thalassaemia (HbA2 of 5.2%). When screened for the eight Common Indian mutations by Reverse Dot Blot, both father and mother showed negative results where as the patient showed a possibility of a mutation in the region covering Codon 15 of the beta globin gene. Further sequencing showed that mother is heterozygous for Codon 15(−T). The patient showed a homozygous pattern for Codon 15(−T) (which is a false positive for homozygous pattern). Codon 15(−T) results in the termination codon at 18th position. To detect the

mutation in the father and the second mutation in the patient, primers flanking the break points of $\psi\beta$, δ, β gene were used. Analysis revealed that both patient and father are heterozygous for Asian Indian type 3 HPFH deletion. There by the patient is compound heterozygous for Asian Indian type 3 HPFH and Codon 15(−T) of beta globin gene. Here by we would like to put forward the significance of HPLC in detection for haemoglobinopathies and to include HPLC as screening test for haemoglobinopathies.

Abstract 131

Pure Herbal Molecule from Indian Medicinal Plants: A New Hope to Overcome the Problem of Drug Resistance

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Nowadays the major problem of leukaemia is the drug unresponsiveness or drug resistance. To overcome this problem we have to identify and prove some small molecules as potential therapeutics against leukaemia. Here we identified two small molecules (one is carbazole alkaloid and another is steroidal lactone) from the two different species of two well known Indian medicinal plants. Both these pure herbal molecules have a potential effect over cell death of lymphoid (B and T) and myeloid (chronic and acute) cancer cell lines and as well as the primary cells isolated from the blood of different lineages of cancer patients. Both the compounds have a superb effect over the in vivo xenograft in athymic nude mice model and reduce the tumor mass effectively. Here we did the toxicity testing and identified that both compounds are minimally toxic towards normal human blood cells and umbilical cord blood derived CD34+ cells. Compounds are also non-toxic towards the non-specific tissues and don't have the adverse effect over body mass at the over optimal dose. On extensive exploration of signalling pathways regulation, we have identified a few pathways targeted by those pure small molecules.

Abstract 132

Prenatal Diagnosis of Thalassemia by Chorionic Villous Sampling and DNA Analysis in 283 Consecutive Couples from Eastern India

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Parents who are thalassemia carriers have 25% chance of getting a thalassemic baby which can be diagnosed in early pregnancy by DNA analysis of placental sample obtained by means of chorionic villous sampling. The birth of diseased babies can then be prevented by medical termination of pregnancy. In our clinic, 283 couples who were known thalassemia carriers were counselled about the prevention of thalassemia by the above method. After informed consent, the diagnosis of thalassemia carrier state was reconfirmed and DNA analyses of parents were carried out. The pregnant mother between 10 and 12 weeks of pregnancy then underwent chorionic villous sampling under ultrasound guidance as day care procedure. The profile of molecular lesions obtained by DNA analysis of parents was as follows: IVS nt 1, 5 in 344 individuals (60.7%); cod 26 in 116 persons (20.5%), FS 41/42 in 22 (3.9%), cod 15 in 21 (3.7%), cod 30 in 15

(2.6%), FS 8/9 in 4 (0.7%), and uncharacterized in 20 (3.5%) of cases. Four pregnancies (1.4%) got aborted as complication of the procedure. In 281 instances, DNA analysis could be carried out. Out of these, 73 pregnancies (25.8%) had to be terminated due to affected foetus while in 2 cases (0.7%) the diagnosis was wrong and baby was born with thalassemia although a carrier foetus was diagnosed. In 208 pregnancies, the diagnosis could be made successfully and healthy babies were delivered.

Abstract 133

Treatment of Severe Hypoplastic Anemia with ATG, Cyclosporine and Methyl Prednisolone

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A total of 10 patients (9 adults and 1 pediatric) with severe aplastic anemia and normal liver and kidney functions were taken up for the study. The investigations done included besides routine bone marrow biopsy and biochemistry, a cytogenetic analysis and flow cytometry for CD55 and CD59 to rule out PNH. After preliminary investigations and written consent, a PICC line was inserted. Then they were started on Methyl Prednisolone 250 mg/day and horse Anti thymocyte globulin (ATG) 40 mg/kg for 5 days. The dose of methyl prednisolone was gradually tapered over the next 21 days. They were started on oral Cyclosporine roughly 2–3 days after stopping ATG. After 6 months of therapy and follow up, a total of 5 patients (50%) showed complete remission. Three patients (30%) had partial remission while one pediatric patient (10%) showed no response whatsoever. One patient was lost for follow up and is counted as a failure.

Abstract 134

Prevalence of Rare Bleeding Disorders in India: Study from a Tertiary Care Centre

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Background Rare bleeding disorders (RBD) are autosomal recessive diseases. This includes the inherited deficiencies of coagulation factors such as fibrinogen, factor (F) II, FV, FV + FVIII, FVII, FX, FXI, FXIII and multiple deficiency of vitamin K dependent factors. Clinical manifestations range from mild to severe bleeding tendencies. They represent 3–5% of all the inherited coagulation disorders with the prevalence of 1 in 500,000, being higher in areas where consanguineous marriages are diffuse. There is a limited available data on the prevalence of RBD in India. The natural history and spectrum of clinical manifestations of RBDs are not well established, since few centres in the world have the opportunity to see a significant number of these rare patients. This study was initiated to evaluate the prevalence of individual disorders, clinical features and phenotype analysis. *Methods* Patients referred for complete haemostatic work up with bleeding history were included in the study. Cases of RBD were segregated from a period between January 1998 and August 2010. Demographic details (Age, Sex) and patients details were included. Phenotype analysis—The combined use of the coagulation tests prothrombin time (PT), activated partial thromboplastin time (APTT) and thrombin time (TT) is usually used to identify RBDs, Specific assays for each different coagulation factor was done to determine the

level of deficiency (severe, moderate, mild). Immunoassays to measure the conserved antigen levels are not strictly necessary for diagnosis and treatment but were done for dysfibrinogenemias and FXIII deficiency. *Results* Data on 326 patients were analyzed, distribution of RBD were found to be 30.7% of FXIII deficiency, 13.2% of FI, 12.6% of FV, 12.3% of FX and FV + FVIII, 11.6% of FVII, 4.3% of FII, 3% of FXI deficiency (Table 1). Mucocutaneous bleeds were common in all rare bleeding disorders, which includes epistaxis, gum bleeds, post traumatic prolonged bleeding. At least one episode of severe bleeding manifestation was associated with FX, FXIII, FI (Afibrinogenemia) and FV. Among 9 FXI deficient, 5 had no bleeding history and rest 4 had mild to moderate bleeding tendency. *Conclusion* Even though there is a paucity of data on the prevalence, clinical manifestation, and genotype analysis of RBD's, this study could be a preliminary data for prompt diagnosis, prophylaxis, or treatment that may significantly improve the quality of life in these subset of patients. Awareness of this disorder amongst physicians, especially in the setting of consanguinity and characteristic symptoms may help in increased and early detection of cases.

Table 1 Classification of RBD based on laboratory phenotype

RBD	Mild	Moderate	Severe
FI	17 (>50 mg/dl)	14 (10–50 mg/dl)	11 (<10 mg/dl)
FII	5	4	5
FV	29	5	3
FVII	25	5	4
FV + FVIII	29	13	0
FX	28	7	2
FXI	0	0	9
FXIII	0	2	79

Abstract 135

Hepatitis: A Silent Threat Among Healthy Blood Donors

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Background Blood transfusion is a life saving intervention. However, like all treatments, it may result in acute or delayed complications. In particular, it carries the risk of Transfusion Transmissible Infections (TTIs) including HIV, Hepatitis B and C, Syphilis and malaria among other less common ones. Hazards of transfusion can be minimized by proper screening and donor selection before collection of blood. Blood donors are considered to be a healthy population. However, over the years, there has been an alarming rise of transfusion transmitted infections especially hepatitis (B&C) among blood donors. *Aims of the Study* This study was done to find out the incidence of Transfusion Transmitted diseases (TTD's) among the blood donors in our hospital. *Methodology* A 5-year retrospective study (2004–2009) of seroreactive cases of TTDs among blood donors was done in the Blood Transfusion Unit of a tertiary care hospital in North India. Data were retrieved from the records maintained in the transfusion service. *Results* A total of 64,528 donors were screened for TTDs (HBV, HCV, HIV 1 and 2, Malarial parasite and Syphilis) of which majority were males. Seroprevalence for transfusion transmitted infections was 2.7%. Seropositivity for Hepatitis was 2.4%. Of all patients with Hepatitis, 1.4% were reactive for HCV infection followed by HBV (0.9%), HIV 1 and 2 (0.2%), Syphilis (0.1%) and Malaria (0.02%). *Conclusion* There has been a significant rise of seroprevalence of

hepatitis, especially HCV among the blood donor population which is considered to be a healthy population. Measures need to be taken at the national level to combat this alarming trend.

Abstract 136

Spectrum of Lymphomas in a Single Reference Laboratory in Eastern India

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The study has been carried out on 100 consecutive cases of lymphomas diagnosed by immunohistochemistry in Drs Tribedi & Roy Diagnostic Laboratory, Kolkata between November 2008 and May 2009. The cases were referred by different hospitals of the city. The objective of this study was to find out the spectrum of lymphomas in this part of the country. Due to lack of adequate immunohistochemistry facility, information regarding spectrum of lymphomas in this area is not available. The present study reveals that diffuse large B cell lymphoma (DLBCL) is the most common type of lymphoma and constitute more than 50% of all cases of Non Hodgkins lymphoma (NHL). The incidence of Hodgkin lymphoma (HL) is about two times that of western population. Follicular lymphoma constitutes only 15% of NHL. However, the incidence of T cell lymphomas is comparable to that of western population.