Tuesday, July 29, 2014

Poster Session: 9:30 AM - 5:00 PM

Hematology/Coagulation

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Clinical Utility of Hematological Parameters to Predict Sepsis Prior to Clinical Presentation in Medical Intensive Care Unit Patients

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Introduction: Sepsis is characterized by pathogen invasion into the bloodstream and the host's response to this invasion. Early detection of sepsis allows for rapid initiation of therapy, decreases morbidity and mortality, and reduces healthcare expenses.

Objective: To investigate the diagnostic utility of hematological parameters to predict the onset and severity of sepsis in Medical Intensive Care Unit (MICU) patients prior to clinical presentation of systemic inflammatory response syndrome (SIRS).

Methods: This retrospective cohort study employed 125 MICU patients with SIRS. These patients were identified by software that scans electronic medical records and alerts investigators when a MICU patient meets SIRS criteria. Several hematological parameters were quantitated on the Sysmex XE 5000 analyzer from specimens collected 24 or 48h prior to the patient meeting SIRS criteria. Procalcitonin was quantified in residual plasma using the Vidas B.R.A.H.M.S® PCT assay (bioMerieux, Inc). The diagnoses of non-infectious SIRS, sepsis and sepsis severity were blindly adjudicated by 2 MICU physicians as: SIRS (n=70) and Sepsis (n=55; severity: sepsis n=7, severe sepsis n=22, and septic shock n=26). Receiver operator characteristic (ROC) curves were generated to evaluate the diagnostic utility of these hematological parameters to predict sepsis.

Results: Areas under the ROC curves (AUC) for each parameter to predict sepsis or severe sepsis/septic shock are listed in table 1. Four hematological parameters WBC, ANC, % neutrophils, and IGC, were significantly different between septic and non-septic patients and between patients with early/no sepsis vs. severe sepsis or shock. Additionally, WBC, ANC, and % neutrophils showed significantly improved diagnostic strength to predict sepsis when compared to a sepsis biomarker, procalcitonin.

Conclusions: Certain hematological parameters measured before onset of overt symptoms of systemic inflammation accurately predicted patients who developed sepsis, severe sepsis and shock. The diagnostic utility of these markers may be improved by combining them into logistic regression models.

Table 1: Diagnostic Utility of Selected Hematological Parameters Measured 24 - 48 Hours Prior to Onset of SIRS to Predict Sepsis and Sepsis Severity

	Sepsis vs. No Sepsis			Early/No Sepsis vs. Severe Sepsis and Shock		
Predictor	**AUC**	**95% CI**	**P value**	**AUC**	**95% CI**	**P value**
Procalcitonin (PCT)	0.67	[0.57-0.76]	0.001	0.69	[0.60-0.79]	<0.001
White Blood Cell Count (WBC)	0.7	[0.61-0.79]	<0.001	0.68	[0.58-0.77]	<0.001
Red Blood Cell Count (RBC)	0.52	[0.42-0.62]	0.74	0.53	[0.43-0.64]	0.54
Immature Granulocyte Percentage (IG-%)	0.60	[0.50-0.70]	0.07	0.61	[0.51-0.71]	0.04
Immature Granulocyte Count (IGC)	0.66	[0.57-0.76]	0.001	0.66	[0.57-0.76]	0.002
Neutrophils (Neut %)	0.72	[0.63-0.81]	<0.001	0.74	[0.65-0.83]	<0.001
Absolute Neutrophil Count (ANC)	0.71	[0.62-0.80]	<0.001	0.71	[0.62-0.80]	<0.001
Platelet Count	0.52	[0.41-0.62]	0.74	0.52	[0.42-0.63]	0.64

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Comparison of coagulation factors assays in two automated platforms

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Background: The determination of coagulation factors II, V, VII and X in the plasma is indicated to diagnosis congenital or acquired deficiency factor, to distinguish dysproteinemias, to aid in therapeutic monitoring of concentrated prothrombin and oral anticoagulants and to verify the function of protein synthesis in the liver. This study aims to evaluate the analytical performance of factors II, V, VII and X measured in Siemens BCS XP System in comparison with Stago STA Compact System performance.

Methods: In a normal population, 18 samples (n = 18) were collected with citrate as anticoagulant. Within 4 hours after collection, samples were analyzed by coagulometric method simultaneously on Siemens BCS XP System, using optical detection and Siemens deficiency factor plasmas and on STA-Compact using mechanical detection. In order to establish correlation between methodologies, we evaluate coefficient of correlation, slope and intercept.

Results: Statistical data is summarized in the table below.

Analytical performance comparison for deficient plasmas						
Assay	**n**	**Regression eq.**	**r**	**Mean**	**SD**	**Selection range**
Factor II	18	y=0.3996x + 66.885	0.64	94	18	67% - 133%
Factor V	18	y=0.6791x + 13.734	0.94	109	23	72% - 153%
Factor VII	18	y=1.2714x – 35.975	0.91	120	24	78% - 217%
Factor X	18	y=0.7043x + 20.576	0.90	123	21	87% - 155%

Conclusion: We observed good correlation between platforms for the V, VII and X factors. For Siemens BCS XP, matrix of factor II is of human origin, providing better performance and low correlation with Stago platform on which the matrix is a mixture of human serum and bovine plasma.

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Plasma cell myeloma with rare presentation

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Background: Plasma cell myeloma is a multifocal plasma cell neoplasm associated with an monoclonal immunoglobulin called M-protein in serum and/or urine. The disease spans a clinical spectrum from assyntomatic to aggressive forms and due to deposition of abnormal immunoglobulin chains in tissues. The diagnosis is based on a combination of pathological, radiological and clinical features. Over 90% of cases of mieloma occur in patients 50 years old or older. The median age at diagnosis is 70 years. Only 2% of cases begin before the age 40.

Objective: The objective of this case is to bring awarness to atypical presentations of plasma cell myeloma that may hinder its diagnosis. In the present case the difficulty in diagnosis is due to the atypical age at onset, absence of monoclonal immunoglobulin in the protein electrophoresis, hypocelularity of bone marrow without typical elements of the pathology and presence of histiocytes and plasma cells with cytoplasmatic inclusions.

Clinical case: CLMS, 38 year old woman, addmited to Hospital Paulistano due to metrorrhagia and pelvic pain. CBC: hemoglobin 5.6 g/dl, platelets: 86,000/ mm^3. Radiographic study: lytic lesions in the hip. Blood marrow count : hipocelularity of all myeloid cells; 1.6% of plasma cells with abnormal morphology; presence of cells with crystallized cytoplasmic imunoglobulin with a lamellar pattern. Protein electrophoresis with absence of M-protein. Blood Immunofixation with presence of monoclonal kappa without correspondence with heavy chain IgA, IgG or IgM. Urinary Immunofixation: presence of monoclonal kappa without correspondence with heavy chain IgA, IgG or IgM. Bone marrow biopsy with imunohistochemistry report: CD68 clone PG-M1 positive in nomerous histiocytes with crystallized cytoplasmic imunoglobulin. Kappa positive in most plasma cells with large cytoplasm. CD 138 positive in about 40-50% of cells. FDG-PET: hypermethabolism in the lithic lesions of the iliac crest. Increased FDG uptake in the bone that corresponds to bone marrow activity.

Conclusion: The enlarged lamelar cytoplasm of plasma cells and histiocytes, probably correspond to the cytoplasmic accumulation of immunoglobulin. The identification of these cells as plasmocites, associated with the imunohistochemistry, FDG-PET and blood and urinary immunofixation allowed the diagnosis of Plasma Cell Myeloma of Kappa chain.

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Validation of Thrombin-Antithrombin III Complex by Enzyme-Linked Immunosorbent Assay in Humans, Non-Human Primates, and Canine Citrated Plasma to Support Pre-clinical and Clinical Coagulation Studies

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Background: The conversion of prothrombin into active thrombin is a significant event within the coagulation cascade. Thrombin is primarily inhibited by antithrombin (ATIII) in which results in a stable inactive proteinase/ inhibitor complex. The concentration of thrombin-antithrombin III complex (TAT) can be measured, and represents a sensitive clinical biomarker for the diagnosis of thrombotic disease in the coagulation cascade.

Methods: Siemens Enzygnost® TAT micro immunoassay (Catalog #:OWMG15) was validated in human, non-human primate (NHP), and canine citrated plasma. The concentration of TAT was measured in-vitro quantitatively through a sandwich enzyme immunoassay and used two different antibodies directed against thrombin and ATIII, respectively. The first incubation step consists of TAT binding to peroxidase-conjugated antibodies that are attached to the surface of the microtitration plate against thrombin. The second incubation step consists of a reaction in which the enzyme-conjugate antibodies are bound to the free ATIII determinants. Any unbound constituents and excess enzyme-conjugated antibodies are removed by a series of washes after each incubation. The enzymatic reaction between hydrogen peroxide and chromogen is stopped by the addition of diluted sulphuric acid. This results in a color intensity change which is proportion to the concentration of TAT. TAT concentrations are measured photometrically through the SPECTRAmax 384plus reader within the kit standard concentration range of 2 to 60 µg/L. For higher TAT concentrations, the sample was diluted using Sekisui TAT deficient material (Catalog #: 203) for humans and normal TAT levels (<4 µg/L) for NHP and canine.

Results: Intra-assay was established through 5-10 replicates and the %CV was ≤4.6 for human, ≤10.8 for NHP, and ≤8.3 in canine. Inter-assay precision was established through a minimum of 3 separate assay runs and the %CV was ≤1.50 for human, ≤15.5 for NHP, and ≤11.4 in canine. The limit of blank was established by analyzing 10 replicates and determined to be 0.135 µg/L for human and 1.3 µg/L for NHP. The lower limit of quantitation was determined as 2.36 µg/L with a %CV of 11.28 in human and 2.10 µg/L with a %CV of 15.7 in NHP. The upper limit of quantitation was determined as 60.15 µg/L and %CV of 4.58 in human and 60.0 µg/L and %CV of 13.4 in NHP. Dilutional linearity was determined using samples near the upper limit of the assay calibration curve, and spiked recovery was determined using samples spiked with several concentrations of kit calibrators. All results were within 20% of the expected value. Sample freeze/ thaw stability was performed using samples with concentrations near the lower and middle limits of the assay calibration curve and consist of 4 freeze/thaw cycles. All results were within 20% of the expected value. Sample stability was performed for human and canine samples frozen at -80 °C. Human samples were stable up to and including 3 months, and canine samples were stable up to and including 2 weeks.

Conclusion: All outlined criteria for the validation of TAT in human, NHP, and canine were met and used to support pre-clinical and clinical coagulation studies.

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Inevitable is fasting time for coagulation laboratory tests - Preliminary evaluation

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Background: Errors in the preanalytical phase generate further work or additional investigation that may cause unnecessary procedures for patients. This study was aimed to evaluate the inevitability of fasting time for coagulation laboratory tests.

Methods: The first blood sample was collected from 10 healthy volunteers at fast (12h). Immediately after blood collection, the volunteers consumed a standardized meal (Table 1).

Table I. Nutritional composition of standardized meal

Nutritional composition	Slice of cheese	Yorgut	Slice of bread	Chocolat snack	Fruit juice	Total
Number (overall weight)	1 (25 g)	1 (125 g)	2 (46.8 g)	1 (20.7 g)	1 (200 g)	417.6
Kcal	64	134	126	105	134	563
KJ	266	562	532	438	572	2370
Protein (g)	4.4	4.1	4.2	1.1	0.8	14.6
Carbohydrate (g)	0.8	19.4	22	12.7	32	86.9
Sugar (g)	0.8	N/A	3	10	N/A	13.8
Total lipids (g)	4.6	4.4	2.4	5.5	0	16.9
Saturated lipids (g)	3.1	N/A	0.8	3.7	0	7.6
Fibre (g)	0	N/A	0.9	0.2	2	3.1
Sodium(g)	0.3	N/A	0.206	0.02	0	0,606
Calcium(g)	0.133	0.131	N/A	N/A	N/A	0.264

The following blood samples were collected 1, 2 and 4 hours after the end of the meal. Each phase of sample collection was standardized, including use of needles and vacuum tubes of the same type and lot. Coagulation tests included the following: activated partial thromboplastin time (aPTT sec), prothrombin time (PT sec), fibrinogen (mg/dL), antithrombin III (AT %), protein C (PC %) and protein S (PS %). The significance of differences between samples was assessed by paired Student's t-test after checking for normality by the D'Agostino-Pearson omnibus test. The level of statistical significance was set at $p < 0.05$.

Results: One hour after food intake, variations were observed for PT (-2.3%, P=0.45) and AT (1.9%,P=0.04). Two hours after meal differences were observed for aPTT (-4.0%, P=0.03) and PT (-3.8%,P=0.57). Statistically significant increases could be observed four hours after the meal only for AT (3.0%,P=0.02). The results of fibrinogen, PC and PS were not influenced by the meal at any time points.

Conclusion: Significant variations of aPTT, PT and AT coagulation laboratory tests after a standardized meal were observed. In conclusion these preliminary outcomes had shown that the fasting time should be carefully considered when performing these tests, in order to prevent spurious results and reduce laboratory errors especially in the therapeutic monitoring.

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Mean platelet volume in patients with pre-eclampsia

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Background Larger platelets have greater haemostatic efficiency than smaller ones by producing larger amounts of vasoactive and prothrombotic components. Mean platelet volume (MPV) is a useful marker indicating alteration of platelet activity which shows association with various inflammatory diseases. Pre-celampsia (PE) is a disease characterized by endothelial damage, elevated intravascular platelet activation while increased MPV in the second of third trimester of pregnancy has been reported. The aim of this study is to evaluate possibility of MPV as a marker of recovery from PE or eclampsia after delivery.

Methods Twenty-one pre-eclamptic and one eclamptic women who gave birth at Kyung Hee Medical Center during January 2011 to June 2012 were include in the study. The results of white blood cells (WBC) count, hemoglobin (Hb) concentration, platelet (PLT) count, and MPV were obtained using an automated hematologic analyzer, Advia 2120 (Siemens Diagnostics, Tarrytown, NY, USA). Medical records were analyzed retrospectively. Postpartum laboratory data of 16 patients with existing serial results of the day of delivery, a day after and two days later were analyzed retrospectively.

Results Nineteen patients had preterm delivery and three had term delivery. Major comorbidities of diabetes mellitus (DM) and myoma were presented in eight patients. Both MPV and platelet count were shown to have decreasing tendency over the observed period, although statistical significance was not shown (P=0.152 and P=0.327, respectively). A ratio of MPV/PLT was slightly increased without statistical significance (p=0.222). Longitudinal serial data of six postpartum days in 5 pre-eclampsia women showed definitely decreasing tendency.

Conclusions MPV has shown decreasing tendency during postpartum periods following PE or eclampsia. These results demonstrate reduced platelet activity and decreased maternal intravascular systemic inflammation after resolution of pregnancy. This study provides evidence that MPV is able to reflect the recovering state from PE or eclampsia in postpartum periods. Most of the previous

studies were focused on increased MPV at pregnant period of PE. Meanwhile, we carefully suggest MPV could be supportive surrogate marker as recovery from inflammatory state after delivery in PE based on these results of our study. Large scale prospective studies are required to affirm these findings and elucidate the underlying mechanism to induce changes of MPV in PE or eclampsia.

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Capillary electrophoresis identification and laboratory evaluation of a complex protein finding in a patient serum

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Background: A 74-years old female was evaluated by her primary care physician, who ordered basic routine tests. All results were within the reference intervals, except the serum electrophoresis, which revealed two unusual intense bands expressed in the gamma region.

The aim of our study was to better characterize the finding according to the guidelines in order to provide more information to the physician.

Methods: Laboratory analysis was conducted by performing a high resolution capillary electrophoresis using the Capillarys® (Sebia) for protein identification. In order to define abnormal protein type, immunofixation of Immunoglobulins and κ and λ light chain, antisera was performed using agarose gel and reagents with the Hydrasys® Electrophoresis System (Sebia). Subsequently, the direct measurement to define the level of immunoglobulins and light chains was performed by nephelometry using BN II® (Siemens Healthcare Diagnostics).

Results: Immunofixation with Immunoglobulins and κ and λ light chain antisera showed the presence of two monoclonal bands against IgG λ and an IgA κ compatible with a biclonal gammophaty. The direct nephelometric measurement revealed a normal IgG level of 1010 mg/dl, a high IgA level of 849 mg/dl, a normal IgM level of 60.5 mg/dl, a high level of κ light chain level of 416.0 mg/dl and λ light chain level of 228.0 mg/dl. The κ/λ ratio of 1.82 was within the reference interval.

Conclusion: In this particular case, the initial diagnosis was apparently a polyclonal distribution, and although not initially requested, the use of complementary tests like quantification and immunological identification, by immunoglobulins profile, were important to help the physician to streamline the diagnosis, monitor and stratify the risk of this biclonal pathology.

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Mean platelet volume (MPV) in patients with chest pain

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BACKGROUND AND OBJECTIVES. Variation in platelets size is indicative of change in platelet function; mean platelet volume (MPV) is directly related to platelet activation in vivo, which plays a central role in the pathogenesis of many vascular diseases. Recent studies have shown conflicting results about the relationship between increasing MPV and cardiovascular disease. This work was aimed at investigating if there is a difference in mean platelet volume between patients with acute myocardial infarction (AMI) and subjects with other cardiac diseases or non-cardiac chest pain, owing to platelets activation in acute coronary syndromes.

METHODS. The study included 5927 consecutive patients with acute non traumatic chest pain presenting to the emergency department of a medium-sized hospital over a period of 30 months, from January 2011 to June 2013. The median age of study participants was 61 years (range, 47 to 93); 3051 (51,5%) were male, 2876 (48,5) were female. EDTA anticoagulated whole blood and serum samples were obtained on admission from all patients. According to the Universal Definition of Myocardial Infarction, AMI was diagnosed in patients showing a rise and/or fall of cardiac troponin I (cTnI) above the diagnostic threshold for MI (0,30 ng/mL), typical changes of electrocardiogram, imaging evidence of new loss of viable myocardium or presence of an intracoronary thrombus. MPV was measured in the course of complete blood count with Sysmex XE-2100 automated hematology analyzer using the Hydro Dynamic Focusing and Direct Current Detection. cTnI was measured by Architect *i* System with chemiluminescent microparticle immunoassay (CMIA) from Abbott Laboratories.

RESULTS AND CONCLUSIONS. AMI was diagnosed in 652 (11%) patients with chest pain (364 men, 288 female). Mean MPV measured at hospital admission was 11.2 ± 0,7 femtoliter (fL) in patients with AMI and 10.4 ± 0,5 fL in all other subjects, with a mean difference of 0.8 fL (95% CI 0.6 – 1.1; P < 0.05). These results show that MPV is higher in patients with acute myocardial infarction compared to those with other cardiac diseases or non-cardiac chest pain, suggesting a substantial role of platelets activation in the formation of the thrombus that occludes the culprit coronary arteries. Therefore, MPV is a potentially useful biomarker of platelet activity in the setting of coronary atherothrombotic events.

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Targeted Metabolomic Profiles Are Strongly Correlated With Metabolic Alterations In Patients With Sickle Cell/Beta Thalassemia Disease

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Background: The complex pathophysiology of Sickle Cell Disease (SCD) makes unlikely that a single therapeutic agent will prevent or reverse all SCD complications. Metabolomic analysis might help in the characterization of the endogenous and exogenous effects of potential new treatments. Metabolites are small molecules that are chemically transformed during metabolism and provide a functional readout of cellular state. Metabolites serve as direct signatures of biochemical activity and are therefore easier to correlate with phenotype. The metabolome is typically defined as the collection of small molecules produced by cells and offers a window for interrogating how mechanistic biochemistry relates to cellular phenotype. There are very few reports providing comprehensive measurements of metabolites present in blood and almost no reports on metabolites changes associated with SCD. In this context we aimed to detect and to quantify targeted metabolites' abnormalities in patients with Sickle Cell/beta thalassemia disease (HbS/βThal) and their implication in pathways that might be of interest to prevent vaso-occlusion and/or to monitor the effects of new therapies on SCD.

Patients and Methods: Thirty adult patients with HbS/βThal were enrolled in the study, while 20 apparently healthy individuals matched for age served as controls. Targeted metabolome analyses based on aminoacids and carnitines were performed after extraction from dry blood spots (DBSs) on filter paper using High Performance Liquid Chromatography followed by tandem Mass Spectrometry (LC/MS/MS), with derivatization (AB SCIEX 5500 triple quadrupole and QTRAP® LC/MS/MS Systems, Framingham, MA, USA) with reagents provided by Chromsystems Instruments & Chemicals, Germany. The injection volume was 10 µL and the analysis lasted ca. 2 min.

Results: The main results of the study showed that: a) fifty metabolites were separated in patients and controls samples, b) mapping the results of analyses, patients with HbS/βThal compared to controls had 17 metabolites with significantly lower concentration, 10 metabolites with comparable concentration and 23 metabolites with significantly higher concentration, c) L-arginine and L-ornithine concentrations were significantly lower in patients HbS/βThal compared to controls, 9.3±2.6 vs 14.7±3.7 µmoles/L, ($p<0.01$), and 116.0±15.0 vs 211.2±19.5 µmoles/L, ($p<0.06$) and d) carnitine, acetylcarnitine and propionylcarnitine correlated significantly positive with reticulocyte production index ($p<0.001$).

Conclusions: Our findings showed significant alterations in whole blood metabolome of patients with HbS/βThal. Also we demonstrated the very important metabolic abnormality of Nitric Oxide biosynthesis pathway due to the low concentration of the aminoacids serving of substrates in this cycle and disturbances in carnitine and acylcarnitines homeostasis. These abnormalities in the metabolome reflected the hemolysis, inflammatory process and pulmonary hypertension observed in these patients.

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Validation of Fluorescence in Situ Hybridization assay for detection of rearrangements involving the Mixed Lineage Leukemia gene

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Background: The Fluorescence *In Situ* Hybridization (FISH) testing has become an important tool of clinical practice in many laboratories dealing with neoplastic deseases. The FISH is more sensitive than conventional chromosomal analysis because can detect a specific alteration in both dividing and nondividing cells and small population of abnormal cells. Although the performance of the most FISH probes has been evaluated by the manufacturer prior to marketing, they also must be validated prior implementation of assay for clinical use. Clinical laboratories must independently adopt protocols for verify the performance of the assay. Rearrangements involving the Mixed-Lineage Leukemia gene (MLL) represent 10% of abnormalities detected in acute leukemias, which in many cases represent poor prognosis. Thus the rapid identification of these rearrangements is needed to guide prognosis and treatment.

Objetive: To validate FISH assay for detection of rearrangements involving the MLL gene following recommendations from the American College of Medical Genetics (ACMG).

Methods: We use the MLL dual-color breakapart probe manufactured by Cytocell®. In the familiarization phase the analysts should become familiar with the probe labeling, testing probe strategy and result reporting. Metaphase cells obtained from 5 karyotypically normal male blood samples were used to localize the probe and determine its analytical sensitivity and specificity. To establish a reference range (normal cutoff) were estimate the false positive rate from 20 uncultured bone marrow samples that would be unlikely to harbor a MLL rearrangement. Two analysts score 500 interphase cells (250 per analyst). All MLL probe signal patterns were recorded. The normal cutoff for each signal pattern was calculated using the BETAINV and CRITBINOM function available in Microsoft Excel.

Results: The MLL breakapart probe presents two portions of the gene, differentially labeled: the proximal 5 'labeled with green fluorophore, the distal 3' labeled with red fluorophore. A typical result of using this probe should show 2 fusions of green and red signals (2F). The separation of the fusion signals indicates the presence of MLL rearrangement. The clinical validation of FISH showed rearrangement involving MLL gene, in agreement with conventional karyotyping. The probe demonstrated 100% specificity and analytical sensitivity. Among the 20 bone marrow samples analyzed, six had one or two false positive cells, which have been designated by the abbreviation 1F1R1G (one fusion, one red, one green). MLL atypical probe signal patterns were also found: 1F, 3F, 1F1G and 2F1G. The cutoffs obtained with BETAINV function was validated for counting 200 cells. The signal patterns and respective normal cutoff are 1F1R1G (2.3%), 1F(4.4%), 3F(1.4%), 1F1G(3.0%) and 2F1G(1,4%). The BETAINV function does not allows a cutoff of zero and show minimal change with increasing cell score. It can minimize the problem of false positive signals that may be due to probe random loss or gain of signals, diffuse probe signals in cells with less condensed chromatin, probe size and design, sample type and others.

Conclusion: The FISH for MLL gene rearrangements with Cytocell ® probe was considered approved.

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Thrombocytopenia In Children with Malaria

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Background: The objective of this study was to access the occurrence and severity of thrombocytopenia in children with malaria at the Child Health department of Korle Bu Teaching Hospital between January and June 2013.

Methods: It was a retrospective study, done at the Child Health Department of Korle Bu Teaching Hospital. Data regarding positive cases of malaria <

12 years attending the Out Patient Department and admitted at the Emergency Room between January 2012 and June 2012 were obtained. Patients were further assessed for thrombocytopenia and its severity. Data were analyzed by Chi square test using SPSS version 13.0.

Results: A total of 131cases were included in the study with a mean age of presentation of 8 years. Plasmodium falciparum was identified in 119 (90.8%) patients while Plasmodium malariae in 6 (4.5%) patients and Plasmodium ovale in 3 (2.3%) with 3 (2.3%) cases or mixed infections of Plasmodium falciparum and Plasmodium ovale.

Thrombocytopenia was observed in 93(71%) cases, of which 40(31%) cases had mild, 56(43%) cases moderate and 34(26%) cases had severe thrombocytopenia. Thrombocytopenia was equally found in falciparum, malariae and ovale infections with no significant difference in severity between falciparum, malariae and ovale species.

Conclusion: Thrombocytopenia is frequently seen in malaria and it is not dependant on type of malaria. In any acute febrile illness, thrombocytopenia should alert one to the possibility of malaria.

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Hemophilia C with a Cys482Trp Mutation in the F11 Gene

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Background: Coagulation factor XI (FXI) is a member of the "contact pathway" and is either activated intrinsically by coagulation factor XII (FXII) or by thrombin, which is produced by an extrinsic pathway and plays an important role in hemostasis. Factor XI deficiency, also known as hemophilia C, is a predominantly autosomal recessive genetic bleeding disorder. The F11 gene encodes the FXI protein, and mutations in the F11 gene have been found in patients with FXI deficiency. We report the first case of a heterozygous mutation (Cys482Trp) in the F11 gene, resulting in a mild FXI deficiency in a Korean patient.

Methods: A 14-year-old male patient with intermittent epistaxis was admitted to the hospital because of increased epistaxis frequency. The patient did not have any abnormal medical history that would have indicated bleeding tendency, apart from being treated for allergic rhinitis. The blood test results were as follows: leukocyte count, 5.6×10^9/L; hemoglobin level, 159 g/L; and platelet count, 215×10^9/L. The blood coagulation test showed normal prothrombin time but prolonged activated partial thromboplastin time (aPTT; 45.9 seconds, reference range, 20.9-35.0 seconds). The level of von Willebrand factor was within the reference range. The activity levels of factors II through XII were within the reference ranges, whereas FXI showed slightly decreased activity (26%; reference range, 60-140%). The patient's genomic DNA was extracted from the collected whole blood sample by using the Easy-DNA Kit (Invitrogen Corporation, Carlsbad, CA, USA). Exons 7, 8, 11, and 13 of the F11 gene were amplified by polymerase chain reaction (PCR). Direct sequencing of the amplified regions was performed by using the ABI Prism 3500dx automated genetic analyzer (Applied Biosystems, Foster City, CA, USA), the same primers that were used to amplify the 4 exons of the F11 gene by PCR, and the Big Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems). To determine whether the patient had a DNA mutation, Sequencher software (Gene Codes Corporation, Ann Arbor, MI, USA) was used to compare the patient's DNA sequences to the reference DNA sequence (GenBank accession number, NM_000128.3). The guidelines of the Human Genome Variation Society (HGVS) were used to identify the sequence variations.

Results: Direct sequencing of the proband demonstrated a heterozygous mutation, c.1500C>G (p.Cys482Trp) of exon 13 in the F11 gene.

Conclusion: The Cys482Trp missense mutation identified in our patient had been previously reported in England, but has never been reported in Korea. Owing to the small number of FXI deficiency cases with associated mutations that have been reported in Korea to date, further studies are warranted to contribute to the development of a database that would help clarify the distribution of mutations present in the Korean population. Such a database would also facilitate studies aimed to clarify the possibility of a founder effect for F11 gene mutations. Furthermore, these efforts would help improve the molecular and genetic diagnostic strategies used for Korean patients.

A-293

The utility of flow cytometry in the diagnosic of hemolytic anemias

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Background: Hereditary spherocytosis (HS) is the most common inherited anaemia in Northern Europe and North America. The red cells in HS show abnormal morphology attributable to a deficiency or dysfunction of one of the red cell cytoskeleton protein (spectrin, ankyrin, band 3 and/or protein 4-2). Recently is used the flow cytometric test that measures the fluorescence intensity of red cells labelled with the dye eosin-5-maleimide (EMA), which reacts covalently on the first extracellular loop of band 3 protein. By this technique a decreased expression of Band 3 in the red membrane surface results in a lower mean fluorescence intensity of EMA. The aim of our study is to differentiate between HS and immune and nonmembrane-associated haemolytic anaemias, such as autoimmune hemolytic anemia (IHA) and Thalassemia (TL).

Method: The EMA-binding test was performed as described by King et al. with minor modifications in 150 controls, 44 HE, 17 IHA and 15 Thalassemia patients, using a BD FACSCanto II flow cytometer.

Results: Significant difference in EMA mean fluorescence intensity (MFI) results was obtained between HE and all of the groups studied (control group ($p<0.001$); IHA (P=

p<0.001), and TL patiens ((p<0.001)). There were differences between IHA and TL patients (p<0.022) while we don't find significant differences between TL patiens vs control group (p=0.533). See figure 1.

Conclusion: Measuring the fluorescence intensity of EMA labeled red cells by flow cytometry could be a powerful tool in the study of hemolytic anemias being a method available in most haematology laboratories. In our case has been shown to be effective for the discrimination of hereditary spherocytosis and IHA versus normal controls and other hemolytic anemias. We found a specific pattern of EMA expression in IHA probably due an increased level of reticulocytes in these patients.

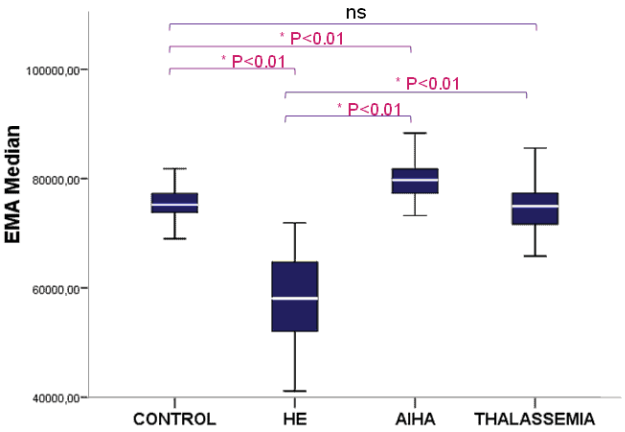

Figure 1: Differential MFI of EMA in the different groups of patients. Data show the mean and standard deviation of EMA. p: statistical significant level (Mann-Whitney U non parametric test for independent samples). MFI: Mean fluorescence intensity. EMA: eosin-5-maleimide. NS: non significant

A-294

Development of An Immunoturbidimetric Assay for the Determination of Haptoglobin Incorporating a New Ready to Use Reagent

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Background: The primary function of the plasma protein haptoglobin is to bind to free haemoglobin thereby preventing haemoglobin-driven oxidative tissue damage, the renal excretion of iron and the subsequent kidney damage following intravascular hemolysis. The plasma levels of this protein are reduced during episodes of hemolysis and the measurements are used in the diagnosis of haemolytic anaemia. Haptoglobin is also a positive acute-phase protein with immunomodulatory properties, the levels of this protein are elevated in inflammatory, infectious processes and in malignancies. This study reports the development of an assay for the determination of haptoglobin in serum samples, which incorporates a new ready to use reagent leading to a simplified procedure and a reduction of handling errors prior to analysis. The assay is applicable to a variety of automated analysers. This is of value for application in clinical laboratories.

Methods: The assay is immunoturbidimetric, the sample containing haptoglobin reacts with anti (human) haptoglobin antibody; insoluble complexes are formed allowing quantitative measurement at 340 nm. The amount of complex formed is proportional to the concentration of haptoglobin in the sample. The reagent is liquid and ready to use. The assay is applicable to a variety of automated systems. Accelerated stability of the reagent was assessed: three lots of reagent in the final packaging were nonstressed (stored at 2^0C to 8^0C) or heat-stressed (at 30^0C, 37^0C and 40^0C for 2, 4, 8, 13, and 26 weeks) and run in parallel. Within-run precision was assessed by testing serum samples at defined medical decision levels, 2 replicates of each sample were assayed 5 times for 1 day. Correlation studies were conducted using a commercially available assay system.

Results: Initial evaluation assigned 56 weeks real time stability for the reagent. The assay showed a sensitivity of 0.19 g/L and was linear up to 3.66 g/L. Prozone was not observed to a concentration of 4.65 g/L. The within-run precision for levels 0.85 g/L and 2.85 g/L, expressed as %CV, was 1.0% and 1.2% respectively. In the correlation study 95 serum patient samples were tested and the following linear regression equation was achieved: y = 1.03x - 0.04; r = 0.999.

Conclusion: The results indicate applicability of this immunoturbidimetric assay to the determination of haptoglobin in serum samples. The inclusion of a new ready to use reagent leads to a simplified procedure and a reduction of handling errors prior to analysis. This is of value for application in clinical laboratories.

A-295

Free Protein S correlation between two systems

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Background: Protein S is a vitamin K plasma protein synthesized by the liver. During blood coagulation, protein S forms a complex with APC which bonds to phospholipids surface and speeds up the proteolytic inactivation catalyzed by APC of the factors Va and VIIIa. The free portion of protein S corresponds to 40% of total protein S. A total or acquired protein S deficiency is associated with a venous thromboembolism risk. There are three types of hereditary deficiencies: type I involves a reduction in total and free protein S levels; type II, a rare form, presents fall in protein S activity and normal levels of total and free protein S; and finally, type III presents normal levels for total protein S and low levels for the free form. Acquired protein S deficiency may be caused by liver dysfunction, nephrotic syndromes and vitamin K deficiency, pregnancy, treatment with L-asparaginase, estrogen therapy, virosis and disseminated intravascular coagulation. This study aims to evaluate the free protein S analytical performance on Siemens BCS XP System compared to obtained by Elite Pró IL system to decide on the introduction of these tests in routine.

Methods: Fresh and frozen blood samples were dosed (n=32), collected with citrate as an anticoagulant. The sample range selection situates between 40% and 122%. Plasma was analyzed within two hours after unfreeze simultaneously in Siemens BCS XP, using Siemens INNOVANCE Free PS-Ag, and IL Protein S, applying the turbidimetric method through optical detection for both systems. Correlation coefficient, slope and intercept were evaluated to examine the correlation degree between the methodologies of this study.

Results: Comparison resulted in a regression equation y = 0.908x + 17.35 and a correlation coefficient r = 0.83. The simple concordance index is 84%, concordance chance 0.63%, kappa index of 0.57, classified as moderate concordance. From five discordant samples, four are very close of a normality value and don't cause, therefore, clinical impact.

Conclusion: We conclude the Siemens INNOVANCE Free PS-Ag analytical performance on Siemens BCS XP is comparable to Elite Pró IL. Thus, these tests were approved for laboratory routine implementation.

A-296

A Dual Monoclonal Antibody Chemiluminescent ELISA for the Detection of Hepcidin-25

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Background: Iron is essential for all metazoans. Hepcidin is the peptide hormone that helps maintain plasma iron homeostasis and is an important clinical biomarker. It is first synthesized as an 84-amino acid precursor which is then proteolytically processed into a 60-amino acid pro-peptide and subsequently into the mature and bioactive 25-amino acid peptide. Hepcidin-25 regulates iron bioavailability by binding to the iron exporter ferroportin, causing its internalization and degradation from the surface of duodenal enterocytes, placental trophoblasts, macrophages and hepatocytes. Dysregulation of plasma iron homeostasis contributes to the anemia associated with chronic diseases such as infection, rheumatoid arthritis, and various cancers. As hepcidin-25 is a key regulator of iron homeostasis, a reliable method to detect hepcidin-25 levels in a blood sample would greatly improve our understanding of how to impact the anemia of chronic disease. Here we report the development of a robust immunoassay to measure hepcidin-25 in a blood sample.

Methods: A monoclonal antibody pair (capture and detection) was characterized and optimized for assay development. The capture antibody was coated onto opaque white 96 well plates while the detection antibody was conjugated to horseradish peroxidase (HRP) using standard immunochemistry methods. Antibody coat concentrations and conjugate detection concentrations were systematically titrated to achieve optimal sensitivity and dynamic range. Numerous iterations of coating and blocking buffers were assayed to further enhance assay manufacturability and consistency. Finally, in-process testing of linearity and precision was conducted to ensure robust performance to the end-user.

Results: The assay is specific for hepcidin-25, and does not cross-react with either hepcidin-22 or hepcidin-20. Linearity testing was performed over multiple kit lots and demonstrated a linear range of 60-6000pg/mL, spanning both normal and disease state levels of hepcidin-25 previously reported. Precision of the assay ranges from 5.0-14.0%CV, with sample recoveries falling between ±20%. The assay was used to test a set of normal and non-hematologic cancer samples. We found that, while a range of hepcidin-25 levels was observed in non-hematologic cancer samples, the assay was able to differentiate between normal and disease state sample populations.

Conclusions: Unlike other hepcidin assays, ours utilizes paired monoclonal antibodies that facilitate the direct detection of hepcidin-25 in a sandwich ELISA configuration. The assay also takes advantage of chemiluminescent detection to improve the sensitivity and stability of signal. Taken together, the data support the utility of this monoclonal-based chemiluminescence sandwich ELISA for the specific detection of hepcidin-25.

A-297

Evaluation of the Newly-Developed Serum Ferritin Measurement System, Point Reader™ and Point Strip™ Ferritin

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Background: Serum ferritin is widely measured to evaluate the iron levels in the body. Point Reader™ (USHIO, Tokyo, Japan) and Point Strip™ Ferritin (ASKA, Kanagawa, Japan), a newly-developed serum ferritin measurement system, is based on the principle of immunochromatography. This system measures serum ferritin concentrations in five minutes and may allow detection of the early iron-deficiency. Therefore, the objective of this study was to evaluate the performance of this new system. **Methods:** Unidentified residual serum specimens with known ferritin concentrations were used. Serum ferritin concentrations were measured using Point Reader and Point Strip Ferritin according to the manufacturer's instructions. The absorbance of phosphate buffered saline was measured to ascertain the sensitivity. The reproducibility of the test was determined by taking 8 replicate measurements of the 3 standards that had low, medium, or high levels of ferritin (12.0, 39.7, and 78.8 µg/L, respectively). For the correlation test, serum ferritin was measured using N-assay LA Ferritin (NITTOBO, Tokyo, Japan) on TBA-40FR Accute (TOSHIBA, Tokyo, Japan). **Results:** The minimum detection limit of the serum ferritin using Point Reader and Point Strip Ferritin was determined to be 10.0 µg/L. In addition, the measurement range of this system was 10.0-100 µg/L as determined from linearity testing. This system has a 3.8% reproducibility in a low control, 6.3% in a medium control, and 6.1% in a high control. We examined the correlation between serum ferritin concentrations obtained using this system (y) and those obtained using TBA-40FR Accute (x) (n = 107). The linear regression equation was $y = 1.07x + 0.84$, and the correlation coefficient (r) was 0.956. **Conclusions:** The newly-developed Point Reader and Point Strip Ferritin measurement system is simple and provides fast measurement of serum ferritin concentrations. This system can be used to assess the iron deficiency during pregnancy as well as in infants and blood donors.

Point Reader™ Point Strip™ Ferritin

A-298

Mathematical Model-based Estimation of Red Blood Cell Clearance Identifies Low Iron States in Patients with Normal Complete Blood Counts

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Introduction The healthy human hematologic system is held in a state of dynamic equilibrium by the carefully regulated processes of (1) cell production, (2) cell maturation in the peripheral circulation, and (3) cell clearance. The resulting steady state is routinely quantified by measurements such as hematocrit (HCT), hemoglobin (HGB), or red blood cell count (RBC). Diseases and other conditions perturbing one of these processes may trigger compensation in the others. For instance, decreasing iron availability will eventually lead to iron deficiency and anemia, but prior to the development of anemia, a decrease in RBC production may be compensated by a delay in RBC clearance. This compensatory delay in clearance will maintain the steady state -- and will also confound our ability to discover the problem because the HCT, HGB, and RBC will remain normal. We hypothesize that an estimate of the rate of the underlying RBC clearance process would serve as a useful screening biomarker of decreasing iron availability, helping to identify seemingly healthy patients whose iron levels are close to abnormal or are already abnormally low. These patients should have iron levels checked and may need to be followed more closely.

Methods We used a mathematical model of RBC population dynamics to infer RBC maturation and clearance rates for patients from routine CBC and reticulocyte counts performed on an Abbott CELL-DYN Sapphire automated hematology analyzer. We first established a normal range for the estimated RBC clearance threshold, defined the probability of clearance as a function of an RBC's volume and hemoglobin content. We identified patients whose CBCs were entirely normal according to existing CBC indices but whose RBC clearance thresholds fell below the 5th percentile of the normal range. We then measured ferritin levels for these patients at the time of their entirely normal CBC.

Results We found that among patients with an entirely normal CBC and a low modeled RBC clearance threshold, 5% had abnormally low ferritin levels, and 19% had ferritin levels that were within 5% of the lower limit of the normal range. Low and low-normal ferritin was 4x more prevalent in this low clearance group than in a control group whose RBC clearance threshold was normal. We also found a statistically significant relationship between the estimated RBC clearance threshold and the ferritin level, with lower clearance threshold associated with a lower ferritin level.

Conclusions Iron deficiency anemia is an early sign of a number of important diseases, such as colon cancer, gastric cancer, celiac disease, and more. Our results suggest that RBC clearance threshold is often reduced, possibly as compensation to maintain desired steady states. This compensation confounds our ability to detect the illness by measuring HCT or HGB. But by using a mathematical model of *in vivo* RBC population dynamics, we can estimate a patient's clearance threshold and identify patients likely to have low ferritin levels. These patients would benefit from direct iron level assessment and further revaluation to identify the underlying cause of the decreased iron availability.

A-300

Distribution of hemoglobinopathies and thalessemias in a Northern Alberta population

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Background DynaLIFEDx is the sole laboratory performing hemoglobinopathy and thalassemia investigations for a catchment area of 2 million people in northern Alberta. Samples for hemoglobinopathy and thalassemia testing come from physician request, immigration medicals, and red cell exchange programs for sickle cell anemia patients and as a reflex test generated by the presence of a hemoglobin variant noted on HPLC analysis for HbA1c. We wished to know the frequency of thalassemia and hemoglobinopathies in our catchment area.

Methods EDTA-anti-coagulated blood samples were analyzed by high performance liquid chromatography (HPLC) using the β thalassemia program on the Bio-Rad VARIANT II. Hemoglobin variants found on HPLC were analyzed by electrophoresis at alkaline and acid pH. A complete blood count (CBC) and ferritin tests were requested as part of the hemoglobinopathy/thalassemia investigation.

Results 5562 thalassemia and hemoglobinopathy investigation were physician requested, 1054 HbS screens were requested and 702 hemoglobin variants fortuitously found on HbA1c analysis were analyzed in the period January 1, 2013 to December 31, 2013. 3438 were interpreted as "normal" and 532 were classified as iron deficient

on the basis of their hematology indices (hemoglobin >120g/l, mean cell volume > 80fL), absence of a hemoglobin variant, replete iron status and calculated Mentzer Index and Discriminant Factor. 322 and 370 were classified as α or β thalassemiaa trait respectively. 357 were classified as S trait, 121 as E trait, 63 as D Punjab trait and 62 as C trait. 22 were classified as Homozygous S, 4 as homozygous HbE and 2 as homozygous HbC and 8 as SC disease. 10 were classified as δβ thalassemia trait and 11 as H disease. The remainder was classified as unusual hemoglobin variant or thalassemia.

Conclusion There is a wide diversity of hemoglobinopathies found in northern Alberta. 11% of the hemoglobinopathies were found as a reflex to HbA1c testing.

A-301

Pediatric Reference Intervals for New Reticulocyte and Platelet Parameters

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Objective: Advances in algorithm development for the Abbott CELL-DYN Sapphire has introduced several new red blood cell parameters describing cell-by-cell size and hemoglobin content for circulating reticulocytes (MCVr, MCHr, CHCr). Reported clinical applications include monitoring RBC production kinetics, distinguishing anemia of chronic disease from iron-deficiency anemia (IDA), and detecting pediatric IDA and erythropoietin-associated functional iron deficiency. Additionally, the reticulocyte channel measures the percentage of platelets that contain residual RNA, reported to correlate with megakaryocytic activity. Potential applications for reticulated platelet (rPLT) values include distinguishing production from consumption and predicting recovery in thrombocytopenic patients. The objective of this study was to determine reference intervals for five pediatric age groups.

Methods: 762 K3-EDTA whole blood surplus samples from previously ordered CBCs were analyzed to collect the reticulocyte channel information. The IRB waived consent for the study samples. Children with clinical and/or laboratory evidence of RBC or platelet abnormalities were excluded. The five age groups included; Group 1 = 1 month - 1 year; 2 = 1 - 3 years; 3 = 3 - 6 years, 4 = 6 - 12 years; and 5 = 12 - 18 years. Reference intervals were calculated (SAS/STAT Software) as per the CLSI guidelines C28-A3C. The non-parametric calculation was used for groups with >120 samples, and the robust method was used for two groups with <120 samples. The RBC indices and platelet count were included in the reference interval calculations to provide a comparison to their reticulated counterparts.

Results: The 95% reference interval limits are in Table 1. Ninety percent confidence intervals for the ninety-five percent upper and lower reference interval limits were calculated, as well (data not shown).

Conclusions: Our results suggest age related trends for the new reticulocyte and reticulated platelet parameters.

Table 1.

Group	1	2	3	4	5
N	52	134	92	216	268
Parameter					
RETC (x10^3/uL)	23.56 - 123.60	18.3 - 101.00	22.58 - 99.86	29.74 - 121.63	27.45 - 105.92
pRETC (%)	0.55 - 3.24	0.41 - 2.24	0.51 - 2.23	0.64 - 2.50	0.55 - 2.21
IRF (fraction)	0.12 - 0.40	0.13 - 0.37	0.10 - 0.30	0.10 - 0.31	0.11 - 0.34
MCV (fL)	74.08 - 101.83	74.13 - 87.56	76.06 - 88.39	78.27 - 91.94	79.62 - 94.29
MCVr (fL)	81.76 - 108.27	80.10 - 100.11	81.78 - 100.51	86.01 - 102.94	89.19 - 107.56
MCH (pg)	24.27 - 35.81	25.14 - 30.22	25.70 - 31.29	26.63 - 32.04	26.59 - 32.51
MCHr (pg)	20.07 - 31.66	22.10 - 30.56	23.38 - 31.50	24.94 - 31.94	25.35 - 32.39
MCHC (g/dL)	32.14 - 36.13	32.55 - 35.77	32.63 - 36.58	32.54 - 36.40	32.48 - 35.97
CHCr (g/dL)	25.57 - 31.39	26.64 - 31.73	27.30 - 32.57	27.51 - 32.17	27.33 - 31.56
PLT (x10^3/uL)	184 - 541	152 - 506	171 - 470	172 - 442	152 - 407
prP (%)	1.31 - 8.16	0.95 - 8.93	0.35 - 6.01	0.26 - 7.33	0.33 - 5.22

A-302

The evaluation of affecting factors on platelet reference ranges in the population of Northeastern China

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Objective: The purpose of this study is to evaluate the clinical laboratory variables, their correlation to platelet count (PLT), the reliability of established reference ranges and their applications in clinical research and diagnostic test.

Methods: This study was carried out in Chang Chun city and its suburban area in China. 3800 cases were selected during year of 2010 to 2012. Inclusive criteria were as following: 1. No medications within one month; 2. No medical history of thrombosis and hemorrhagic disease. Exclusive criteria were: 1. Female patients are at menstrual period; 2. Female who are pregnant; 3.Patients who have medical history of liver disease or hematologic diseases. Patients who met the inclusive criteria were put in questionnaire survey for past medical history and social history. 5 ml of blood was drawn for clinical chemistry analysis including liver function test (to exclude unknown hepatic disease), blood glucose test and lipid panels. Hematology variables include platelet count (PLT), mean platelet volume (MPV), plateletcrit (PCT), and platelet distribution width (PDW). SPSS software has been used for ANAOV analysis. P<0.05 was used for statistical significant testing in this study.

Results: This study showed PLT ranged from 147 -363 K/µl. MPV were from 6.8-10.5 fl. PCT were from 0.13%-0.29%. PDW were from 15,5% -18.4%. These variables were affected by the cholesterol and triglyceride level, particularly in the group of 50 years or older. The correlations analysis indicated that the higher the cholesterol, the higher the PLT, MPV, and PCT. The average platelet count was higher in female than male. Smoking, alcoholic, BMI, blood pressure and blood sugar have not effects on platelet variables.

Conclusion: Based on these case studies and the analysis of clinical chemistry variables, we concluded that diet, blood pressure and social behavior do not have impact on platelet values. Age and high cholesterol cause elevated platelets count. This supports the previous study that high cholesterol causes high coagulation diseases including stroke, myocardia thrombosis and deep vein thrombosis.

A-303

Measurement of Serum Ferritin Concentrations in a Japanese Young Population Using a Newly-Developed System, Point Reader™ and Point Strip™ Ferritin

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Background: Iron-deficiency anemia is usually diagnosed by measuring the level of hemoglobin. However, even if the hemoglobin level is within the reference range, the serum ferritin concentrations may still be <12 µg/L resulting in a latent iron-deficiency state without anemia. Blood donation has a significant impact on the iron levels of the body, especially in blood donors with latent iron deficiency. Therefore, the objective of this study was to evaluate the serum ferritin concentrations in a Japanese young population, using the newly- developed serum ferritin measurement system, Point Reader™ (USHIO, Tokyo, Japan) and Point Strip™ Ferritin (ASKA, Kanagawa, Japan).

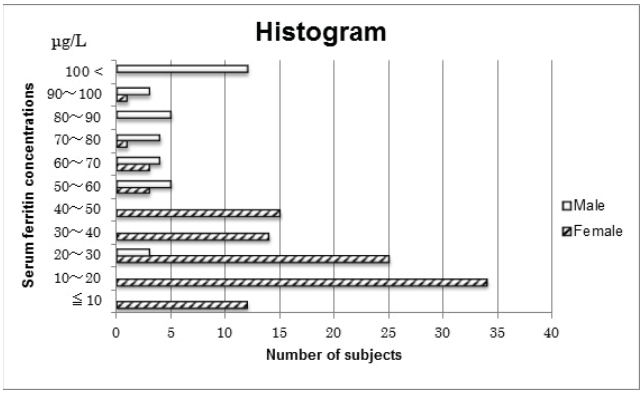
Subjects: Serum and blood samples were obtained from 36 male and 108 female students (age, 20-24 years) from Bunkyo Gakuin University, Tokyo, Japan. The ethical committee of Bunkyo Gakuin University approved this study, and informed consent was obtained from all subjects.

Methods: Serum ferritin concentrations were measured using Point Reader and Point Strip Ferritin based on the principle of immunochromatography. The hemoglobin levels were measured using XE-2100 (Sysmex, Hyogo, Japan).

Result: The serum ferritin concentrations of the young Japanese population were shown in histogram. The serum ferritin concentrations in the 36 male students ranged from 20.2 µg/L to 179.1 µg/L, while that of 12 (11.1%) female students were <10 µg/L. Overall, the serum ferritin concentrations of the male students were observed to be higher than those of the female students. 23 (21.3%) female students showed latent iron deficiency state without anemia.

Conclusions: Out of 108 female students, the 21.3% with latent iron deficiency are at a significant risk of developing iron deficiency anemia from blood loss, such as that

occurring during blood donation. Estimation of the hemoglobin level alone in blood donors may not be adequate; therefore, the estimation of serum ferritin concentrations may also be necessary to detect pre-clinical iron deficiency.



A-304

Prognostic Value of Modest Increases of Plasma D-dimer Concentration in Patients with Previous History of Myocardial Infarction

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Background: D-dimer can be considered as a global marker of the turnover of cross-linked fibrin and of activation of the hemostatic system. We prospectively investigated whether modest increases of plasma D-dimer levels might be relevant to prognosis in patients with a previous history of myocardial infarction. Methods: We studied 606 consecutive patients with a previous history of myocardial infarction (median age, 65 years; 508 males) and estimated glomelurar filtration rate (eGFR) ≥15 ml/min/1.73 m2. Blood samples for measurements of D-dimer, total plasminogen activator inhibitor-1 (tPAI-1) and high-sensitive C-reactive protein (hsCRP) were obtained at enrollment. Among these patients, 65% had hypertension, 36% had diabetes, 35% had chronic kidney disease, and 74% had history of coronary revascularization. Results: During a median follow-up period of 43 months, there were 120 cardiovascular events including 20 cardiovascular deaths. Comparably, patients who had cardiovascular event were older (median, 67 vs. 65 years, p = 0.01), had higher levels of D-dimer (0.65 vs. 0.44 μg/ml, p < 0.0001), and displayed a lower level of eGFR (62 vs. 67 ml/min/1.73 m2, p = 0.02) and left ventricular ejection fraction (LVEF, 50 vs. 53 %, p < 0.0001) than those who did not. On Cox regression analyses multivariate including 12 clinical and angiographic variables, D-dimer levels were independently associated with cardiovascular events (relative risk: 1.92 per 10-fold increment, p = 0.02). Clinical characteristics and cardiovascular mortality and cardiovascular event rates according to tertiles of D-dimer were shown in Table. Conclusion: Modest increases of D-dimer may be independently associated with adverse outcomes in patients with a previous history of myocardial infarction. Measurements of D-dimer may be useful for the risk stratification of adverse outcomes in this population.

	Tertiles of D-dimer (μg/ml)			
	1st < 0.35 (n=208)	*2nd 0.35-0.71 (n=197)*	*3rd 0.71 < (n=201)*	*P value*
Age (years)	*61*	*66*	*70*	*< 0.0001*
Male	*89%*	*84%*	*79%*	*0.005*
Diabetes	*31%*	*38%*	*40%*	*0.08*
eGFR (ml/min/1.73m²)	*73*	*67*	*61*	*< 0.0001*
HsCRP (mg/dl)	*0.05*	*0.08*	*0.14*	*< 0.0001*
tPAI-1 (ng/ml)	*14.6*	*13.8*	*13.7*	*0.42*
LVEF (%)	*54*	*51*	*50*	*0.002*
Cardiovascular event rate	*13%*	*19%*	*28%*	*< 0.0001*
Cardiovascular mortality rate	*1%*	*2%*	*7%*	*0.002*

Data are expressed as median or %.

A-306

Acute erythroleukaemia with atypical presentation

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Background: Acute erythroid leukaemias are a group of acute leukaemias characterized by the predominance of erythroid population. It is divided into two groups based on the presence or absence of a significant myeloid component: Erytroleukaemia (Erythroid/ myeloid) and Pure erythroid leukaemia. Erytroleukaemia (Erythroid/ myeloid) is diagnosed when the presence of erythroid precursors in the bone marrow is ≥ 50% of the nucleated cell population and ≥ 20% of myeloblasts in the non-erythroid cell population. Pure erythroid leukaemia is diagnosed when ≥ 80% of cells in the bone marrow are commited with the erythroid lineage (undifferentiated or proerythroblastic in appearance) and there is no significant myeloblastic component.

Clinical case: MCA, a 71 year old woman was admitted to the orthopedic clinic at Hospital Paulistano with bone pain. Her first blood count showed mild anemia (hemoglobin: 10g/dl). On the course of her hospitalization she presented a severe drop in hemoglobin levels, so a bone marrow count was performed. Bone marrow examination (04/16/2013): Slightly hipocelular bone marrow with 18% of cells from erythroid lineage (5% of those cells were proerytroblasts with morfological alterations). Presence of megaloblasts, binucleated red blood cells and altered cytoplasmic features. Neutrophilic series showing assyncronic maturation, 1% of myeloblastic components and megakaryocytic serie without morphological alteration.

Iron stains: 30% of ringed sideroblasts. Cytogenetic analysis: 45, XX, deletion of the long arm of chromosome 5, terminal deletion of the long arm of chromosome 7, monossomy of chromosome 19 and isochromosome of the long arm of chromosome 21. Bone Marrow examination 07/03/2013: Bone marrow with 50,4% of cells of erythroid lineage with predominance of early forms and 21,6% of myeloid blasts.

Conclusion: This case is an example of a rapidly progressive acute erytroleukaemia, that was initially diagnosed as a myelodysplasic syndrome with 5% of proeriytroblasts and absence of a myeloblasts. The cytogenetic abnormalities found in this patient are present in myelodysplasic syndromes (MDS) as well as in erytroleukaemia. Although there are no specific chromosomic abnormalities, the -5/del, -7/del (both present in this case) and chromosome 8 trissomy are the most common cytogenetic abnormalities found in erytroleukaemia. It is known that the presence of complex cytogenetic abnormalities (more than 3 chromosomal abnormalities) is the only statistically significant independent variable that adversely affects survival in the acute erythroid leukaemia group. The present report shows a case that began with a bone marrow count of MDS with 5% proerytroblasts and absence of myeloblasts and rapidly changed its morfological characteristics to a erytroleukaemia. This case highlights the necessity of a biomarker that can precisely differentiate between these two diseases, so that the appropriate treatment can be initiated without delay.

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Evaluation of the Alcor Scientific iSED Erythrocyte Sedimentation Rate Analyzer

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OBJECTIVE: This study evaluates the performance of the Alcor Scientific iSED analyzer. The analyzer measures erythrocyte sedimentation rate using the principles of hemorheology in a photometric based method.

METHODS: Split sample comparisons were performed over a 5-week time period using the manual Westergren Sedimentation Rate method manufactured by LP Italiana Spa as the predicate device. During the study period two levels of Alcor Scientific control material were run each day that sample analysis was performed.

RESULTS: CVs for the commercial control material run during the study were 29.1% at a value of 3 mm/hour and 7.1% at a value of 35 mm/hour. Studies using fresh patient samples yielded similar results and were maintained over time once the instrument was placed into clinical service. There was no evidence of sample carryover. Correlation between the predicate method results and the iSED analyzer results using patient samples was iSED = 0.7006 x (manual method) + 5.113 with an R^2 value of 0.7412. 217 patients were used in the study (61 males and 156 females). The clinical interpretation of the results for the two methods on all 61 male samples and 150 of the 156 female samples correlated. No pattern or explanation for the 6 female patients with disparate clinical interpretations could be found. For the patient samples the concordance coefficient was 0.9423 (substantial agreement) and the weighted kappa coefficient was 0.9424.

CONCLUSIONS: The analytical performance of the iSED analyzer was acceptable with CVs at least as good as those listed in the package insert. The instrument is easy to operate and requires a short training period. Maintenance is minimal and mechanical performance is acceptable. Although some disparate values were found between the two methods no systematic biases were noted. Instrument repairs are affected via a central service center, so instruments needing repairs must be shipped out to the manufacturer. Loaner instruments are not available at the time of this abstract submission. The downtime associated with instrument repair is unacceptable being in the range of 14 - 20 days. Unless a laboratory has multiple instruments in operation, this represents a significant drawback that needs to be addressed by the vendor.

A-308

Elevated Serum Levels of Von Willebrand Factor Antigen are Associated with Poor Prognosis In Patients with Symptomatic Waldenstrom's Macroglobulinemia

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Background: Waldenstrom's Macroglobulinemia (WM) is an uncommon malignancy which, is characterized by the infiltration of the bone marrow by lymphoplasmacytic cells, which produce a monoclonal IgM. Recently, it was shown that high levels of von Willebrand Factor antigen (vWF:Ag), are associated with adverse prognosis in patients with symptomatic WM and it was suggested that vWF:Ag levels may reflect interactions between lymphoplasmacytic cells and other cells of their microenvironment such as mast cells and endothelial cells. We aimed to evaluate the prognostic importance of vWF:Ag levels of patients with symptomatic previously untreated WM, in order to validate vWF:Ag as a possible prognostic marker for progression free (PFS) and overall (OS) survival.

Patients and Methods: The analysis included 42 patients with symptomatic WM, and 19 healthy controls of matched gender and age. Anemia (250U/L and 58% had serum albumin <3.0g/dL. Median serum IgM was 3340mg/L (range 246-9563mg/dL). According to IPSSWM, 22% had low, 43% intermediate and 35% high risk WM, respectively. Reasons to initiate therapy included cytopenias in 42% of patients, B-symptoms in 15%, hyperviscosity in 12%, neuropathy in 10% and other reasons in 21%. Primary therapy based on rituximab was given in 93% of the patients and 54% achieved at least 50% reduction of IgM. vWF:Ag levels were measured in serum collected before initiation of therapy by means of a latex particle-enhanced immunoturbidimetric assay (ACL Top 3G, Instrumentation Laboratory, USA).

Results: Median serum level of vWF:Ag was 101U/dL (mean 132.5U/dL, range 19.9-399.0U/dL) and were slightly higher compared to the serum levels of healthy controls (median 85.0U/L, mean 85.0 U/L, range 48.0-124.0U/L). However, 6/42 (14%) had vWF:Ag levels median value was more frequent in patients with beta2-microglobulin >3.0 mg/L (p=0.006) and less frequent in patients with low (11%) vs. patients with intermediate (59%) or high (62%) risk IPSS (p=0.036). There was no correlation of vWF:Ag levels with IgM levels or with the extent of bone marrow infiltration or with other manifestations of the disease. Median follow up of symptomatic patients was 4 years. Patients with vWF:Ag levels within the upper quartile (i.e. vWF:Ag ≥200.0U/dL) had a median progression free survival of 12 months vs. 63 months of patients with vWF:Ag <200.0U/L (p<0.001), while the median survival for patients with vWF:Ag ≥200.0U/dL was 37 months (4-year survival 29%) vs. 4-year survival of 97% for patients with vWF:Ag <200.0U/L (p<0.001).

Conclusion: In conclusion, the serum levels of vWF:Ag provide significant prognostic information in patients with symptomatic WM and patients with levels ≥200.0IU/dL have a very poor prognosis compared to patients with lower levels. vWF:Ag measured in the serum, may become an important prognostic marker in WM and needs further validation.

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Full Automation of Heparin Induced Thrombocytopenia ELISA Assay on Dynex DSX ELISA platform.

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Background: Heparin induced thrombocytopenia (HIT), with or without thrombosis, is an important cause of morbidity and mortality in patients treated with unfractionated heparin. HIT is invariably characterized by antibodies specific to platelet factor 4 (PF4) and heparin complex. Our goal was to fully automate a HIT ELISA assay (Immucor, Inc., Norcross GA) with the Dynex DSX instrument (Dynex Technologies, Chantilly, VA) for application in clinical laboratories to achieve a less labor intensive approach and high through-put HIT testing.

Method: 37 patient samples were analyzed side by side using the manual HIT ELISA and the automated HIT ELISA on Dynex DSX. Patient samples were added to micro wells coated with platelet factor 4 (PF4) and complexed to polyvinyl sulfonate (PVS) as a PF4:PVS complex. If an antibody was present and recognizes a site on the PF4:PVS complex, binding would occur. Unbound antibodies were washed away and an alkaline phosphate labeled anti-human globulin reagent (Anti-IgG, A, M) was added to the wells and incubated. The unbound anti-IgG/A/M was washed away, and the substrate PNPP (p-nitrophenyl phosphate) was added. After an incubation period, the reaction was halted with a stopping solution and the optical density (OD) was measured by a spectrophotometer at an absorbance of 405 nm using a reference filter of 490 nm. The only changes in the procedure during the programming of Dynex DSX software from the manual HIT ELISA protocol were the removal of the pre-soaking step prior to pipetting reagent and the programming of a longer incubation time which was extended from 30 to 35 minutes. 20 known negative samples for HIT and 17 known positive samples for HIT were analyzed for the presence of PF4 antibodies by the fully automated method on the Dynex DSX, and then manually run by hand. An optical density (OD) greater than 0.4 is used as positive cut-off for PF4 (HIT) antibodies detection.

Results: The qualitative comparison of results obtained with the manual HIT assay and the automated HIT assay showed 100% agreement using the 0.4 OD cut-off. The correlation using the OD from samples was excellent (y(automated)=0.9x(manual)-0.02, r=0.96) with a bias of -0.1. The precision (between days) using the Dynex DSX was above manufacturer expectation (CV<20%) as CV at low level (OD≤0.3) was 19.5% (n=8) and at high level (OD≥1.8) CV was 5.2% (n=8). Precision using manual procedure was 5.5% (n=6) at OD≤0.3 and 16.2% (n=6) at OD≥1.8. The DSX instrument uses disposable pipette tips and performs all pipetting steps for reagents, controls, patient samples as well as washing and rinsing steps. This method is less labor intensive and improves the turnaround time.

Conclusion: The fully automated DSX instrument for HIT testing showed an excellent correlation with the manual procedure. The HIT assay on the Dynex DSX takes less than two hours. It is a fully walk-away solution once the instrument is loaded with reagents and patient specimens. To our knowledge, this is the first fully automated clinical laboratory HIT assay has been described using the Dynex DSX ELISA instrument.

A-310

New policy reduces manual slide reviews on platelet tests

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Implementation of a new platelet manual slide review policy in a small hospital/clinic laboratory serving an oncology service can produce a modest reduction in slide reviews related to platelet count flags, improve turnaround time, and save an estimated $3/slide review (6 min/case). The policy at this institution for performing a Wright stained slide review on specimens tested using a Sysmex XE-2100 Hematology Instrument was <100,000/uL and/or a Sysmex platelet flag (platelet clumps CLP, platelet abnormal distribution PAD, platelet abnormal scattergram PAS). Absolute delta check values set at Δ20,000/uL (<100,000/uL) and Δ50,000/uL (101,000 - 10^6/uL) reflexed a review of clinical history, but not slide review. The goal of the policy revision was to decrease slide reviews related to platelets on blood samples from patients with known histories of low platelet counts. Real time review of LIS/EHR patient information was a necessary component for the new platelet slide review policy and designed as follows:

•No flags- report result

•Slide review performed first time for thrombocytopenia (<100,000/uL), CLP, PAS or PAD; always for CLP flag

•If TCP <100,000/uL and/or PAD, PAS flags present on hemogram:

-Previous slide review performed, same platelet flag, and patient result review consistent with clinical history, finalize result

-Previous slide review performed and patient result review inconsistent with clinical history, perform slide review, then finalize result

-Previous slide check performed and different platelet flag, perform slide review, then finalize result

Three month's platelet count data collected after implementation of the policy yielded 22 patient platelet test results (60% oncology service patients), 0.4% of 5564 platelet count tests showing platelet flags. Nineteen patients had previously documented platelet counts <100,000/uL within a 4 year time span archived by the LIS. Eighty-six subsequent slide checks were avoided by implementation of the policy. On EHR review of patient records, no significant clinical findings appeared to have been missed by the new policy. Median slide check time, defined as start to finalizing result, was 6 min/case; $3/platelet slide check (savings based on $30 labor and benefits). If this sample reflects our patient population and provider ordering patterns, annual savings are modest at approximately $1000/yr. More important are the improved turnaround time of at least 6 min for these specimens, significant given that average turnaround time for platelet count at this laboratory without flags (receipt to verify) is 10min, and decreased technologist disruption by eliminating unnecessary platelet slide checks. Implementation of a new platelet manual slide review policy modestly reduced the number of manual slide reviews performed on patients showing repetitive blood counts with thrombocytopenia <100,000/uL, abnormal platelet distribution or scattergram flags.

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Can Neutrophil/Lymphocyte Ratio be Used in the Differential Diagnosis of Abdominal Pain of Appendicitis and Familial Mediterranean Fever?

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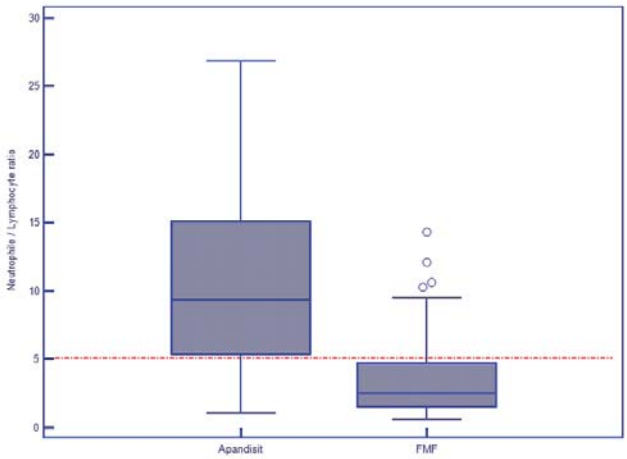
Backround: The neutrophil/lymphocyte ratio (NLR) is proven to be associated with some conditions such as chronic inflammation in cardiovascular diseases, malignancies, ulcerative colitis, gangrenous appendicitis, and amyloidosis. Familial Mediterranean Fever (FMF) is an inherited disorder which is common among individuals of Mediterranean descent. Both FMF and appendicitis may manifest with abdominal pain which is hard to differentiate. This retrospective study aimed to evaluate the ability of the NLR to predict acute appendicitis pre-operatively and to differentiate cases with abdominal pain due to FMF and acute appendicitis.

Methods: A hundred patients who were admitted to emergency unit of Marmara University Pendik E&R Hospital with abdominal pain were included. Fiftysix patients had the diagnosis of appendicitis, were treated operatively and proven as appendicitis histopathologically. FMF group was formed by 44 patients who were monitored at the Rheumatology Clinic with colchicine treatment and without any other diseases.

Results: Median ages (25-75 percentile) of appendicitis and FMF groups were 12 yrs (10-15,75) and 13 yrs (9-17), respectively which were not different significantly (p=0.691). Complete blood counts were measured by LH 780 (Beckman Coulter, USA) and NLR was obtained from the records. Mean NLR values (25-75%) were significantly higher for appendicitis group when compared to the FMF group [2.54 (1,5-4,8) vs. 9.34 (5,32-15,45)] (p<0.001) (Fig 1). The diagnostic performance of NLR to differentiate acute appendicitis from FMF was assessed with a ROC plot and cut-off value of NLR was >4.97. (AUC:0.842, 95%CI: 0.763-0.920, p<0.001) (sensitivity 80% and specificity 77%).

Conclusion: According to the results of our study, NLR of 4.97 seems to be a reliable parameter in discriminating abdominal pain of acute appendicitis from FMF attack. NLR is cost effective, readily available, and can be calculated easily.



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Reticulated Platelets - Towards A Standardized Approach: Results From 'Apparently Disease-free' Subjects In 3 Countries

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Background Despite obvious potential as a sensitive marker of thrombopoietic activity, the lack of a reference method and standard materials for Reticulated Platelet (RP) counting have hampered widespread adoption. Here we applied a new, highly standardized method (described in a separate poster) to assess typical values in healthy ('apparently disease-free' subjects) at 3 international sites. Our goal was to test whether 3 different laboratories, using the same protocol on 3 different flow cytometers, could recover statistically similar results in apparently disease-free ('normal') subjects.

Methods The 3 sites (instruments) were 1) Abbott Hematology, California (Accuri-C6), 2) Liverpool Hospital, Australia (FACSCanto) and 3) London Health Sciences, Canada (Gallios). K_2EDTA specimens were collected from 71 consented voluntary participants in the Abbott staff donor program, lab staff or surplus CBC specimens from subjects without any hematological abnormality. Briefly: fresh (< 8 hours post draw) whole blood was incubated with the CD61-APC/CD41-APC monoclonal antibody mix and stained with TO before fixation with formaldehyde. A second tube, with PBS in place of TO, served as the control. During analysis, a positive marker was drawn on a TO histogram (gated on the platelet cluster) of the control tube to include 0.1% of the negatives. The instrument settings and this marker were held fixed for the acquisition and analysis of the tube containing the TO-stained platelets. Basic statistics (means, medians, SD and CV) were generated in MS Excel 2010, Analysis of Variance (ANOVA) was used to assess the mean results (males, females, pooled) across the 3 sites.

Results The mean RP results from the 3 sites were as follows: California 5.2% (SD 3.3%, n=21), Canada 4.8% (SD 0.7%, n=21) and Australia 4.6% (SD 3.0%, n=29). Mean for the pooled 71 subjects was 4.9% (SD 2.7%, Range 1.7-17.1%). Differences in the means between the 3 sites were not statistically significant ($p > 0.05$). At one site (California) the mean female RP count was higher (6.1%) than the mean male value (4.1%) though the differences were not statistically significant.

Conclusion We have demonstrated that a standardized flow cytometric protocol, at 3 geographically distinct sites, on 3 different instruments, yielded comparable results in 'apparently disease free' subjects. The major shortcoming of all nucleic acid based methods is the non-specific uptake of the nucleic acid dye by platelet dense and alpha granules. We were able to minimize this by using a dilute TO solution. To further mitigate non-specific dye uptake, we used a simple and objective control strategy (negative control tube) to differentiate specific from non-specific staining. We believe this method is simple and robust enough to form the basis for development of a potential reference method. Further studies to define the ideal dye concentration and characterize inter-lab performance are planned.

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Proteomic profiling of platelets in acute ischemic stroke patients

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Background: Platelets are important in the pathogenesis of stroke and ischemic stroke is high level of mortality. Especially antiplatelet agents are useful for prevention and treatment in this patients. Platelets are so easily activated by different stimulants in the circulation which can be affected by activation and apoptosis.

Methods: Using LC-MS based protein profiling, we examined and correlated the proteomic response of stroke patients platelets. Patients were admitted to neurology department who had ischemic stroke within 24 h. Stroke is defined as rapidly developing clinical symptoms/signs of cerebral dysfunction lasting more than 24 h without any cause other than a vascular abnormality. Venous blood was drawn from all patients who had not taken any antiplatelet drugs for the prior 14 days, and was collected into acid citrate dextrose containing tubes for platelet isolation. Platelets tryptic peptides were analyzed in triplicate on the LC-MS/MS system (UPLC-ESI-qTOF-MS). Tandem mass data extraction were done with ProteinLynx Global Server v2.5 (Waters) and searched with the IDENTITYE algorithm against the reviewed database of homo sapiens (www.uniprot.org). Protein Identification detected with PLGS 2.3 and Quantification of the protein expression changes was done with Progenesis LC-MS software V4.0 (Nonlinear Dynamics). In addition, ELISA was used to quantify pro-inflammatory cytokines as TNF-α in the plasma.

Results: Proteomic profiling of platelets obtained from the stroke patients resulted in identification of 500 proteins. Of these, 83 proteins were found to be differentially expressed in patient as compared to control. These differentially expressed proteins were involved in various processes such inflammatory response, cellular movement, immune cell trafficking, cell-to-cell signaling and interaction, hematological system development and function and nucleic acid metabolism. Plasma levels of TNF-α increased in the stroke patients (29.12 ± 1.37 pg/ml) compared with control subjects (5.16 ± 2.84 pg/ml).

Conclusion: This is the first report providing a global proteomic profile of platelets from stroke patients. Our data provide an insight into the proteins that are involved as platelets inflammation response during ischemic stroke. Inflammation caused by stroke changed to platelet cellular proteins interactions in patients.

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Investigating lipid effects on protein C and thrombin-activatable fibrinolysis inhibitor activation by thrombin/thrombomodulin complex

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Background: Thrombomodulin (TM), an endothelial membrane protein, plays central roles in maintaining haemostasis and preventing inflammation by increasing protein C (PC) and thrombin activatable fibrinolysis inhibitor (TAFI) activation by thrombin. APC selectively inactivates coagulation factors Va and VIIIa, thus preventing excessive coagulation. TAFIα modulates inflammatory mediators and reduces plasmin generation. During cell apoptosis, membrane lipids, such as phosphatidylserine and ethanolamine, are enzymatically flipped to the cell surface. Endothelial cell damage often involves lipids changes. The lipid membrane facilitates TM-enhancement of protein C activation by thrombin, however, lipid effect on TAFI activation is unknown. We studied the effects of different phospholipids in generating APC and TAFIα by the TM/thrombin complex in an effort to better understand thrombotic and inflammatory processes for possible implications in diagnosis and treatment monitoring.

Methods: To study the capacity of TM/thrombin to generate APC and TAFIa, liposomes containing TM were generated by extrusion after TM was mixed with a swelled lipid solution of different compositions in Tris-HCl buffer. Liposomal TM was separated from free form by size exclusion chromatography using Sepharose CL-4B. Liposomal TM concentration was verified by first separating lipid and protein components followed by protein content determination by Bradford assay. APC and TAFIα were generated by incubation with liposomal TM and thrombin in otherwise identical conditions. Concentrations of APC and TAFIα were determined by hydrolysis of Spectrozyme PCa and hippuryl-arginine, respectively.

Results: It was shown that incorporation of TM into phoshatidylcholine vesicles increased generation of APC (26.4 pmol ± 0.7 pmol) in comparison to free TM (18.2 pmol ± 0.7 pmol). Phosphatidylethanolamine had decreased APC generation (10.6 pmol ± 0.6 pmol), phosphatidylserine had a large increasing effect (35.9 pmol ± 1.3 pmol) while APC generation by thrombin alone and with phosphatidylcholine vesicles (but no TM) was below detection range. In addition, free TM was added to phosphatidylcholine liposome solution and APC was generated (20 pmol). This data suggested that separate lipid components do not increase APC level as much as the complex between lipids and TM does.Increase in TAFIα generation was observed after TM was reconstituted in phosphatidylcholine (8.34x104 U, where 1 U = hydrolysis of 1 µmol of substrate per min.) and phosphatidylserine containing (9.18x104 ± 0.3x104 U) vesicles when compared to free TM (6.46x104 U). Phosphatidylethanolamine reconstitution resulted in slight elevation of TAFIα, albeit high variation was observed between measurements (7.54x104 ± 1.6x104 U).

Conclusion: We Investigated lipid effects on protein C and thrombin-activatable fibrinolysis inhibitor activation by thrombin/thrombomodulin complex. We found that phosphatidylserine-bound TM dramatically increases the generation of APC and TAFIα in liposome-based systems. This is contrary to phosphoethanolamine, which had a reducing effect on APC generation. This study suggests possible significance of the effects of cell membrane lipids on hemostatic balance and inflammation and could have possible implications related to damaged endothelium situations such as septic shock. Future studies will test this observation with human endothelial cell lines.

A-318

Establishment of reference intervals for HbF and HbA2 in a Northern Alberta population

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Background DynaLIFEDx is the sole laboratory performing hemoglobinopathy and thalassemia investigations for a catchment area of 2 million people in northern Alberta. Samples for hemoglobinopathy and thalassemia testing come from physician request, immigration medicals and as a reflex to the presence of a hemoglobin variant noted on HPLC analysis for HbA1c.A review of our data showed that 17% of HbF results exceeded the manufacturer's reference interval of <1.0% so it was necessary to reevaluate the reference interval for Heft. A reference interval for HbA2 was calculated at the same time. **Method** 6616 thalassemia and hemoglobinopathy investigation requests were analyzed in the period January 1, 2013 to December 31, 2013. 3306 were on patients older than 2 years and interpreted as "normal" by a Clinical Chemist based on their hematology indices (hemoglobin >120g/l, mean cell volume > 80fL), absence of a hemoglobin variant, replete iron status and calculated Mentzer Index and Discriminant Factor EDTA-anti-coagulated blood samples were analyzed by high performance liquid chromatography (HPLC) using the β thalassemia program on the Bio-Rad VARIANT II. Total imprecision (CV%) for HbF was 2.2% and 1.2 % at levels of 2.17% and 9.54% respectively. For HbA2 the total imprecision (CV %) was 3.7% and 2.1% at levels of 2.82% and 5.74% respectively. The statistical software package MedCalc® version 11.4.2.0 for Windows was used to analyze the data. **Results** 3306 individuals, median 31 years, range 3 to 92 years were included in the reference interval calculation. As recommended by CLSI a non-parametric method was used to calculate the reference interval as the Kolmogorov-Smirnov test showed that the distribution of HbF and HbA2 values did not follow a normal distribution. Reference Intervals are HbA2 Lower limit (90% CI) 2.3% (2.20 to 2.34), Upper limit(90% CI) 3.4% (3.3 to 3.45); HbF Lower limit (90% CI) 0.2%(0.20 to 0.23) and Upper limit(90% CI) 1.8% (1.80 to 1.90). **Conclusion** The appropriate reference intervals for the Northern Alberta population using the β thalassemia program on the Bio-Rad VARIANT II was up to 1.8% for HbF and up to 3.4% for HbA2.

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Protein C Antigen And Activity In Patients With Sickle Cell Anaemia

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BACKGROUND: Sickle cell disease (SCD) is characterized by vaso-occlusive events and organ damage which form important causes of morbidity and mortality. Protein C is a naturally occurring anticoagulant that has been demonstrated to be deficient in sickle cell anaemia patients and the impact of hypercoagulable states and thrombosis on sickle cell disease still remains uncertain.

AIM AND OBJECTIVES: The objective of this study was to determine the protein C (PC) levels of the sickle cell patients and to explore the relationship between protein C levels and vaso-occlusive events as well as related complications.

SUBJECTS AND METHODS: A cross-sectional study comprising sixty one sickle cell subjects and thirty healthy control subjects. Protein C antigen (quantitative) and

activity (qualitative) levels were assayed using enzyme linked immunosorbent assay and the Protac (photometric) method respectively. Haematological parameters were measured with the haematology automated analyzer. Data were analyzed using SPSS version 16.

RESULTS: The adult SCD subjects had lower values for both PC antigen (68.6 ± 16.0 %; p=0.004) and activity (49.0 ±13.0 %; p=0.005) when compared to the adult control group (PC antigen = 84.8 ± 18.0 %; PC activity = 60.31±10.37 %). Similarly the paediatric SCD subjects had lower PC antigen (54.9 ± 14.9 %; p=<0.001) and activity (48.0 ± 13.1 %; p=0.006) levels compared to their control group (PC antigen = 79.6 ± 16.7 %; PC activity = 58.6 ± 8.0 %). However there was no significant association between PC levels and SCD complications or vaso-occlusive events (p >0.05).

CONCLUSION: There is functional PC deficiency in SCD patients. This supports a hypercoagulable state in the patients. However there was no significant association between PC levels and SCD

complications or vaso-occlusive events. Thus protein C level in SCD patients may not be a good prognostic marker for disease severity.

PROTEIN C SERUM LEVELS

	Adult			**Paediatric**		
Variables	**SCD (n = 30)**	**Controls (n = 15)**	P-value	**SCD (n = 31)**	**Controls (n = 15)**	P-value
Protein C Antigen Mean ± SD (%)	68.6 ± 16.0	84.8 ± 18.0	**0.004**	54.9 ± 14.9	79.6 ± 16.7	**<0.001**
Protein C Activity Mean ± SD (%)	49.0 ± 13.0	60.3 ± 10.0	**0.005**	48.0 ± 13.1	58.6 ± 8.0	**0.006**

***t-test**

A-320

Thrombocytopenia in Plasmodium falciparum Parasitized Pregnant Women

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Background: Malaria infection during pregnancy is a major public health problem in tropical and subtropical regions of the world. Hematological changes associated with malaria in pregnancy are not well documented, and have focused predominantly on anemia.

The aim of this study was to determine the impact of Plasmodium falciparum parasitaemia on the platelet count of pregnant women at the maternity department of Korle Bu Teaching Hospital, Ghana

Methods: This case control study evaluated the effect of malaria parasitemia on the platelet count of Sixty (60) plasmodium parasitized pregnant subjects. Sixty non- malaria parasitized pregnant women and sixty non-pregnant and non-malaria-infected subjects served as control. 1.0 ml of blood sample was taken into EDTA bottle for Full Blood Count using Mindray BC 5300. Thick and thin film prepared and stained with Giemsa for the determination of P. falciparum parasite species and density using light microscopy. A platelet count of 100 × 109 /L was the threshold at two standard deviations below the mean for healthy Ghanaian pregnant women used to indicate thrombocytopenia. Differences in platelet counts were compared based on malaria species and parasitemia in matched non-pregnant and pregnant women.

Results: The mean platelet counts (×109 /L) were significantly lower in pregnant subjects with an episode of Plasmodium falciparum malaria 101.3 ± 9.2 × 109 /L compared to non-parasitized and healthy non-pregnant controls (245.09 ± 23.10 and 260 ± 50.5 × 109 /L) respectively. Platelet count values were 102.5 ± 9.58 ×109 /L and 116.3 ± 15.7 × 109 /L for the primigravidae and multigravidae respectively. (χ= 10.36; P = 0.05). Parasite density was significantly higher among Plasmodium falciparum parasitized primigravidae compared to multigravidae 2140 (1628-2652) parasites/µL in primigravidae women compared to 1816 (1420-2212) parasites/µL in multigravid women. The mean parasite count in Plasmodium falciparum parasitized subjects was 2610 ± 224 parasites/µL, 95% confidence interval (2082-3108). Malaria parasite was found to exert a significant reduction in platelet density in parasitized subjects. This reduction was more pronounced in primigravidae and multigravidae. An inverse relationship was established between parasite density and platelet count (y =-0.020 ×+86.2, r =-0.3).

Conclusion: There is need for a strengthened antenatal care system with increased awareness of the problem among communities most affected by malaria. Preventative strategies including regular chemoprophylaxis, intermittent preventative treatment with antimalarials and provision of insecticide-treated bed nets should be implemented as well as integration of malaria control tools with other health programmes targeted to pregnant women and newborns.

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Risk Stratification and Progression follow-up of MGUS Patients: value of the sFLC and Heavy Chain/Light Chain Pairs markers

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Background: MGUS is the most frequent MG, usually considered a benign MG, and defined by a serum monoclonal protein (MP) <3 g/dL, less than 10% of plasma cells in the bone marrow, no related organ or tissue impairment and no evidences of other B-cell proliferative disorder. The rate of progression towards Multiple Myeloma (MM) is of 1-2% however, the designation itself highlights the current difficulty in precisely identifing patients that will progress towards malignant MG. Based on the MP size and type and the serum Free Light Chains (sFLC) ratio at diagnosis it is possible to stratify the patients according to probability of progression allowing a better management of the MGUS patients, according to the IMWG guidelines. We have been studying the frequency of specific immunoglobulin heavy/light chain pair's alterations in a cohort consisting of MGUS patients with different risk of progression. To validate the significance of our preliminary findings we have increased the cohort in all risk groups and included the follow-up of patients that have progressed to MM.

Methods: 308 samples from 248 MGUS patients were included, both newly and previously diagnosed. All patients were risk stratified according to the IMWG guidelines serum M-protein levels and type by SPE and IF, and sFLC and HLC by nephelometry (Freelite™ and Hevylite®, respectively). Total immunoglobulin levels were also determined to establish the frequency of classic immunoparesis (BNII. Siemens).

Results: The Hevylite assay allows the determination of an imbalance on the immunoglobulins of the same isotype (i.e., IgG-K/IgG-L ratio) identifying the presence of a MP in serum. It also allows to observe the immunoparesis within the same isotype of immunoglobulin (i.e., suppression of the uninvolved HLC pair IgG-L in a IgG-K monoclonal gammopathy). The frequency of HLC immunoparesis is significantly superior to the classic immunoparesis for low-intermediate (p<.0005) and intermediate-high risk groups(p<.001). Besides, the frequency increases in the higher risk-of-progression groups, although only significantly for the HLC immunoparesis type(p<.01 HLC vs p<.27). In IgM cases the differences between classic and HLC immunoparesis did not reach significance, possibly due to the size of the population. Furthermore, for IgG MGUS patients, both the HLC ratios and the uninvolved HLC immunoglobulin levels show a significant trend towards more extreme values as the risk of progression increases. 3 IgG MGUS patients with low-intermediate risk progressed towards MM. For these particular cases an abnormal sFLC ratio was the only established criteria that indicated the risk for progression: 1) While normal at presentation, the HLC ratio became abnormal during follow-up, initially due to the suppression of the uninvolved HLC pair; 2) both FLC and HLC ratios were abnormal prior conversion and those abnormalities became more extreme with active MM; 3) HLC normal at presentation, the patient evolved into an oligosecretory disease.

Conclusion: The sFLC ratio is a relevant indicator of risk for progression in this population of MGUS patients. The frequency and distribution of HLC alterations (both ratio and HLC-immunoparesis) within the specific MGUS risk-groups is suggestive of its utility as a marker for progression.