Haematology

Cod: 0668

COMPARISON OF THE PERFORMANCE OF THE NEWLY DEVELOPED IGG HEAVY CHAIN/ LIGHT CHAIN IMMUNOASSAYS WITH SERUM PROTEIN ELECTROPHORESIS FOR MONITORING IGG MULTIPLE MYELOMA PATIENTS

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BACKGROUND: International guidelines recommend both serum protein electrophoresis (SPEP) and total immunoglobulin assays (tIg) as tools to quantify monoclonal immunoglobulins (M-Ig). However, SPEP may be inaccurate at low (<10g/L) and due to dye saturation at high (>20-30 g/L) M-Ig concentrations. tIg by contrast is an accurate test but is unable to distinguish between monoclonal and polyclonal immunoglobulins which limits its sensitivity at concentrations approaching the normal range. Newly available assays quantifying heavy/light chain immunoglobulin pairs may offer a solution to the limitations of the recommended assays. Here we compare the performance of these assays, alongside traditional measurements as tools to monitor MM patients.

METHODS: HLC IgGκ and IgGλ concentrations were measured nephelometrically in 127 MM (87 IgGκ, 40 IgGλ) patient sera. Results were compared to published normal ranges (IgGκ: 4.03-9.78 g/L, IgGλ: 1.97-5.71 g/L, IgGκ/IgGλ: 0.98-2.75), historic SPEP and immunofixation results; Weighted Kappa and Pearson correlation were used to analyse results.

RESULTS: At presentation, all 127 patients had a quantifiable band by SPE (median (range): 38 g/L (11-82)). Similarly all 127 patients had an abnormal HLCr and involved HLC (iHLC) concentrations (median (range) IgGκ: ratio 56 (6-1275), iHLC 32 g/L (14-102); IgGλ: ratio 0.024 (0.001-0.329), iHLC 34.5 g/L (9.3-90.3)). Pearson correlation indicated a good agreement between SPE and iHLC concentrations at presentation and during follow up ($y=0.83x+1.8$, $R^2=0.87$). A good agreement was also shown between the changes in SPE and iHLC concentrations during follow up ($y=0.87x-0.05$, $R^2=0.83$). Weighted Kappa analysis showed substantial agreement between the responses assigned by either changes in SPE and iHLC (81% agreement, Weighted Kappa (95% CI): 0.78 (0.56-1.00)) or changes in SPE and HLCr (71% agreement, Weighted Kappa (95% CI) 0.74 (0.56-0.92)).

CONCLUSIONS: The recommended monitoring methods are limited to sensitivity and technical issues. The HLC immunoassays provide an alternative method of quantifying M-Ig in patients with MM.

Haematology

Cod: 0669

PERFORMANCE EVALUATION OF COMPLETE BLOOD COUNT ON AUTOMATED HEMATOLOGY ANALYZERS

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BACKGROUND: This study was designed to determine the correlation between hematological parameters by Mindray BC5500, Medonic CA620 automated hematology analyzers and micro pipette adapter (MPA) device of Medonic CA620 hematology analyzer.

METHODS: Sixty (60) subjects were randomly selected from both apparently healthy subjects and those who have different blood disorders from the Agri Military Hospital, Agri, Turkey. 2 mL of venous blood sample was collected from each subject into di-potassium ethylenediamine tetra-acetic acid (K_2EDTA) for the analysis of hematological parameters. Blood samples were obtained from these tubes to capillary tubes to analyse via MPA. All the blood samples were tested automatically for white blood cell (WBC), red blood cell (RBC), platelet (PLT), hemoglobin (HGB), mean corpuscular volume (MCV), mean cell hemoglobin (MCH) and mean cell hemoglobin concentrations (MCHC). All the data were statistically analyzed with EP Evaluator (version 8.0.0.171) program.

RESULTS: The results showed that there were statistically significant differences in PLT counts and HGB values between MPA and the other automated analyzers. However, the magnitudes of these differences were not clinically significant. Also, MCHC and MCH values were significantly different between Medonic CA620 and the other two methods. There were no statistically differences in WBC, MCV and RBC values between three methods.

CONCLUSIONS: From the present study, it can be concluded that the automated hematology analyzer readings correlated with each other. Besides, it is seen that, analysis of blood samples with MPA device using capillary tubes for whole blood parameters is a reliable and acceptable alternative to automated analyzer.

Haematology

Cod: 0670

ERYTHROCYTE AND RETICULOCYTE INDICES BY SYSMEX XN ANALYSER IN HEMODIALYSIS PATIENTS

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BACKGROUND: Reticulocyte hemoglobin content and percentages of hypochromic red cells have been proposed as accurate measures of iron status and reliable markers for monitoring therapy's effectiveness in the European Best-practice guidelines. We investigate the reliability of reticulocyte hemoglobin equivalent (RetHe) and percentages of hypochromic red cells (%HypoHe) reported by Sysmex XN analyser (Sysmex Corporarion, Kobe, Japan) in predicting the response to iron administration in hemodialysis patients (HD).

METHODS: forty HD patients were studied, 17 females and 23 males (35-83 years, mean 70.3. As most patients received IV iron, administration was interrupted at least 3 weeks before the study. Two samples were analysed for each patient: a baseline after the not receiving iron supplement period; and a second sample, four weeks after IV iron administration. Hemogram including reticulocyte indices were obtained within 6 hours of collection. Serum ferritin, serum iron and transferrin saturation were analysed. Responders were defined as patients who had an hemoglobin increase of at least 10 g/L compared with baseline. Differences between responders and non-responders were evaluated by Student t test and $p < 0.05$ was considered significant. To identify the efficiency of the test and the optimal cutoff for predicting the response to iron administration, receiver operating characteristic analysis (ROC) was performed.

RESULTS: according to established criteria, 21 patients were responders and 19 non responders. Non responders Ret He mean was 32.8 pg (SD 2.7 pg) while responders was 30.5 pg (SD 1.7 pg). Non responders HypoHe% mean was 1.7% (SD 2.0 % pg) while responders was 6.9 % (SD 4.4 %). Both indices means were statistically different ($P<0.0001$) between the two groups.
ROC analysis results: RetHe Area under curve(AUC) was0.84 (95%CI 0.64-0.93), cut off 30.8 pg, Sensitivity 78.7 % and Specificity 87.2 %; %HypoHe AUC was 0.78 (95%CI 0.64-0.91) cut off 2.4 %, Sensitivity 72.2 % and Specificity 88.1 %

CONCLUSIONS: %HypoHe and RetHe provide direct information on iron availability. Both are reliable parameters for the study of erythropoiesis status in HD patients and useful to guide therapy.

Haematology

Cod: 0671

COMPARISON OF D-DIMER TESTING WITH IMMUNO-TURBIDOMETRIC AND ELFA METHOD. WHICH METHOD IS USEFUL FOR OUR LABORATORY?

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BACKGROUND: D-dimer (DD) is formed by plasmin, via the destruction of the fibrin clot which is constituted with the cross-links and the activation of coagulation system due to any reason. Although, ELISA is accepted as gold-standard for DD measurement method; turbidimetric immunoassays which are faster and less complex compared to the ELISA, indicated equal susceptibility with ELISA and showed somewhat better selectivity than ELISA in the patients of emergency service. Thus, in this study we purposed to evaluate methodological and clinical compatibility between ELFA (Enzyme-dependent fluorescence technique) and immuno-turbidimetric method which are used in DD measurement.

METHODS: 168 citrated plasma samples that are sent to biochemistry laboratory within 11 days for DD analysis are studied with immuno-turbidimetric ELFA method (ELISA, DD exclusion kit, bioMerieux Vidas®) which is present in our hospital laboratory and with immunoturbidometric method (BCS analyzer, İnovance DD kit, Dade Behring) which is planned to be recruited. Clinical inconsistency between these two methods is compared with Passing-Bablok regression analysis and Bland Altman method. Difference between dependent groups is examined with Student t test.

RESULTS: When the results of the studies which are performed with these two methods on total 168 patient samples are compared, R value is detected as 0,986.Regression equation is found as BCSDD=1,0341(%95 CI, 0,9854 to 1,0924)* Vidas DD - 9,5220(%95 CI, -39,7899 to 21,2956). For both of the two methods, in 164 out of 168 samples (97.6%) permitted error limits are found. Method is found as successful. In t test with the dependent group it is detected that two tests do not differ from each other ($p>0.05$).

CONCLUSIONS: In our study , between both of these two methods statistically sufficient and meaningful correlation is detected. While making a choice between these two methods, institutions should consider some factors such as; additional sample requirement, amount of the sample, the duration of result giving, linearity of the test, additional device/equipment and personnel requirement.

Haematology

Cod: 0672

THE RELATIONSHIP BETWEEN LEUKOCYTOSIS AND PLATELET PARAMETERS

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BACKGROUND: In recent studies, the role of platelets in thrombosis, immunity, inflammation and angiogenesis have been investigated, and association between thrombosis and inflammation and platelet parameters has been investigated as well. The aim of this study was to investigate the relationship between platelet parameters, including platelet count, mean platelet volume (MPV), platelet distribution width (PDW), and plateletcrit (PCT), and white blood cell (WBC) count in conditions resulting in leukocytosis.

METHODS: Blood samples form 206 volunteers were analyzed for platelet parameters and WBC counts using Coulter LH 780 Hematology Analyzer (Beckman Coulter Inc.). We evaluated all platelet parameters and WBC counts in all samples, with the WBC counts being above reference upper limit (10.000/μL). The statistical analyses were performed for evaluation of the results.

RESULTS: The study showed the mean ± SD values as 260.000 /μL ± 105.000 for PLT count, 8.24 fL ± 1.6 for MPV, 16.93 % ± 0.71 for PDW, and 0.20% ± 0.07 for PCT, all which were within reference ranges. On the other hand, there were no statistically significant correlations between the WBC counts and platelet parameters (P>0.05).

CONCLUSIONS: One can say on the basis of the findings that no relation of WBC count to PLT counts and related parameters is present in leukocytosis conditions. However, for each leukocytosis condition, the individual platelet parameters may be evaluated.

Haematology

Cod: 0673

COMPARISON OF NON-INVASIVE VS. CAPILLARY AND VENOUS HEMOGLOBIN DETERMINATION IN VOLUNTARY BLOOD DONORS

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BACKGROUND: Pre-donation hemoglobin (Hb) screening is a mandatory test for selection of donors for blood donation. Today there are various methods of Hb estimation with its own advantages and limitations. In addition to standard invasive methods which are based on determining the concentration of hemoglobin in the sample of venous or capillary blood, it is also used a non-invasive method which is based on occlusion spectroscopy technology in the red/near-infrared range. At the core of this technology is the generation of strong optical signal, resulting from temporary blood-flow occlusion made by a pneumatic finger cuff. The main objectives of this study were to compare three different methods for Hb determination in blood donors and to ascertain whether non-invasive hemoglobinometry could replace the traditional invasive methods for Hb determination.

METHODS: This prospective study was conducted on 494 potential blood donors, aged between 19 and 64 years (mean: 39,19±11,07) who have passed the medical examination. Potential blood donors were stratified by sex, weight, number of previous donations, pulse, blood pressure and donation status (accepted/deffered). Pre-donation Hb values were estimated using three different methods: on HemoCue Hb201 (HemoCue, Sweden) from capillary blood, on Beckman Coulter automated cell counter from venous EDTA blood samples, and on non-invasive Hemomatic (LMB Soft).

RESULTS: The Hb values obtained by HemoCue were in range 11,4-18,2 g/dl (mean: 14,76± 1,23 g/dl), with a bias of 0,32 g/dl from the non-invasive Hb determinations (mean: 14,66±1,12 g/dl) and 0,39 g/dl from the Coulter venous Hb values (mean: 14,53±1,09 g/dl). Non-invasive method showed statistically significantly higher negative predictive value than standard invasive methods (82,48% for NMB200, $p<0,0001$ vs. 73,68% for HemoCue and 78,58 % for Coulter, $p<0,05$). On calculating the sensitivity, specificity and positive predictive value of methods there was not statistically significant difference between them.

CONCLUSIONS: Non-invasive hemoglobinometry is a valid method for pre-donation Hb measurement and can be considered as an alternative to standard invasive methods.

Haematology

Cod: 0674

LC-MS/MS ANALYSIS OF PLASMA POLYUNSATURATED FATTY ACIDS IN PATIENTS WITH HOMOZYGOUS SICKLE CELL DISEASE

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BACKGROUND: The aim of this study was to determine circulating omega-6, omega-3 polyunsaturated fatty acids (PUFAs) and prostaglandin E2 (PGE2) levels in steady state sickle cell disease (SCD) patients.

METHODS: Blood was collected from healthy hemoglobin (Hb)A volunteers and steady state homozygous HbSS patients who had not received blood transfusions in the last 3 months. Plasma levels of arachidonic acid (AA, C20:4n-6), dihomo-gamma-linolenic acid (DGLA, C20:3n-6), eicosapentaenoic acid (EPA, C20:5n-3) and docosahexaenoic acid (DHA, C22:6n-3) were determined by an optimized multiple reaction monitoring (MRM) method using ultra fast-liquid chromatography (UFLC) coupled with tandem mass spectrometry (MS/MS). Prostaglandin E2 (PGE2) was measured in serum samples by enzyme immunoassay.

RESULTS: Plasma AA and DGLA were significantly increased while EPA and DHA were significantly decreased in SCD plasma compared to control. Serum PGE2 levels, AA/DHA and AA/EPA ratio was significantly higher in SCD patients when compared to control group.

CONCLUSIONS: The significant increase in PGE2 levels, AA/EPA and AA/DHA ratio confirms the presence of a proinflammatory state in SCD patients.

Haematology

Cod: 0675

DISTINCT CLINICAL CHARACTERISTICS IN MYELOPROLIFERATIVE NEOPLASM WITH CALRETICULIN MUTATIONS

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BACKGROUND: With the recent discovery of somatic insertions/deletions in the calreticulin (CALR) gene in myeloproliferative neoplasms (MPN), the definite molecular diagnostics has become available in >90% of cases with clonal disorder. The aim of our study was to apply a complex array of molecular techniques to identify driver mutations in a large cohort of MPN patients allowing the phenotypic comparison of subgroups with different mutations.

METHODS: A diagnostic algorithm of allele-specific PCR (JAKV617F presence), quantitative TaqMan assay (JAK2V617F quantity), high resolution melting analyzes (MPL mutations), fragment analyzes by capillary electrophoresis and Sanger-sequencing (CALR mutations) was applied in a cohort of Hungarian MPN-patients: 222 polycythemia vera (PV), 283 essential thrombocytosis (ET) and 98 primary myelofibrosis (PMF).

RESULTS: PV patients all carried the JAK2 V617F mutation. In the ET-cohort, the frequency of V617Fmut was 51.9% (n=147), CALRmut 34.4% (n=97), MPLmut 3.2% (n=9), while 10.6% of patients (n=30) were JAK2-CALR-MPL mutation (triple) negative. Similar distribution of the above mentioned somatic mutations was observed in PMF patients: 57.1% V617Fmut (n=56), 24.5% CALRmut (n=24), 7.1% MPLmut (n=7), 11.2% triple-negative (n=11). Comparing CALRmut ET-patients to V617Fmut ET patients, younger age at disease onset (53 vs. 60 years, p=0.03), higher platelet count (981 vs. 775 G/L; p<0.001), lower hemoglobin (131 vs. 146 g/L; p=0,027) and lower white blood cell count (9 vs. 10 G/L; p<0.001) was observed. Venous thrombosis (17.9 vs. 8.1%, p=0.04), arterial thrombosis (15.1 vs. 9.3%, p=0.21) or hemorrhage (9.5 vs. 4.7%, p=0.18) occurred more frequently in V617Fmut compared to CALRmut ET patients, resulting in higher risk for vascular complications (37.2 vs. 18.6%, p=0.003). On the other hand, post-ET myelofibrosis was more frequent in CALRmut ET-patients compared to V617Fmut ET-patients (14.8% vs. 6.2%, p=0.03), while leukemic-transformation occurred with the same frequency (3.4 vs. 2.7%).

CONCLUSIONS: We confirmed that CALRmut MPN is associated with distinct clinical characteristics. The recent discovery of the somatic CALR mutations improve the precision of non-invasive diagnostics of patients with clonal disorder.

Haematology

Cod: 0676

PSEUDOTHROMBOCYTOPENIA IN THE PRESENCE OF LARGE PLATELETS

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BACKGROUND: Macrothrombocytopenias, or large platelets, are found in Werlhof's disease, myeloproliferative neoplasms, MYH-9 gene related syndromes, Alport's syndrome, Bernard-Soulier syndrome, etc. In these cases, real thrombocytopenia, clinical bleeding and other associated pathologies exist. Mediterranean or familial macrothrombocytopenia can also be singled out, a benign hereditary disorder with few platelets but of greater size. Cases exist where macrothrombocytes are present but unassociated with any pathology and the automatic analyser is unable to count them causing erroneous results. It is important to carry out a peripheral blood smear in search of these abnormalities when faced with unexpected false counts even though manual platelet recounts are imprecise. The objective is to detect and correct possible cases of pseudothrombocytopenias, as a consequence of macrothrombocytes, unassociated with any pathology.

METHODS: The equipment used for platelet determination is ADVIA 2120 (Siemens®) which uses laser beam diffraction to carry out the analysis. Recounts are made either using an EDTA hemogram tube or a sodium citrate coagulation tube, obtaining similar platelet counts. We note down the number of platelets obtained by the analyzer; the number of platelets counted using a manual method (Fonio); the mean platelet volume (MPV) and the large platelet morphologic alarm (LPLT).

RESULTS: 48 cases presented where pseudothrombocytopenias were detected owing to the presence of large platelets. Out of these, 40 cases were detected via LPLT with an MPV above the normal value (> 11.1 fl), only 8 cases showed normal MPV (7,2- 11,1 fl) without triggering the alarm. Those with MPVs between 11,1- 11,9 fl set off the alarm: LPLT +; VPM between 12-12,9 set off the alarm LPLT ++; and VPM > 13 fl also set off the alarm LPLT +++. In cases where MPV was normal and there were no alarms, we confirmed platelet anisocytosis through a blood smear, normal platelets coexisting with those of an enlarged size. Manually in all cases the resulting figures were higher than those obtained by the analyser.

CONCLUSIONS: Following detailed observation of the autoanalyzer data, the existence of large platelets can be suspected and, consequently, a blood smear is always necessary for a manual count.

Haematology

Cod: 0677

MALARIA IN THE PONIENTE AREA OF ALMERIA, SOUTHERN SPAIN

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BACKGROUND: Malaria is an endemic disease in the countries of North Africa produced by a parasite of the genus Plasmodium. Five species exist that can affect man: falciparum, ovale, vivax, malariae and knowlesi, being the most common and serious infection caused by P. falciparum. According to WHO, each year there are 219 million cases and 660,000 deaths. In 2012 in Spain there were 484 cases declared. Given the high incidence of immigrants coming to our area from Africa over the last few years, it has become a social health problem. We detail the incidence of malaria in our hospital, in particular during 2013 and analyse the most relevant haematological data.

METHODS: After the clinical suspicion of malaria in a subject recently arrived from endemic zones, we carried out a diagnosis using a blood smear, an immunodiagnostic test and PCR. For cases diagnosed during 2013, we collected demographic data, a record of the time since their return to Spain and haematological parameters.

RESULTS: Since 1998, 157 cases of malaria have been diagnosed. Yet 117 of these cases have been recorded in just the last five years (75%). The year 2013 saw the highest incidence, with 37 cases. Countries of origin include: Mali 22, Senegal 5, Equatorial Guinea 3, Nigeria 2, Guinea-Bissau 1, Guinea 1, Burkina-Faso 1, Gambia 1 and Ghana 1. All of these immigrants, who live in our area, return to their endemic countries to visit family and relatives (VFR), apart from one case of a Spanish citizen who works in Equatorial Guinea. The average age is 34 years old (range 7-50), 35 cases correspond to males. The diagnosis was carried out by blood smear in 32 cases and PCR in 5. 35 infections were from P. falciparum and 2 from P. ovale. The average time back in Spain before diagnosis was 29 days (1-180). 26 cases presented with thrombopenia (70%, N >120,000/mmc in the negroid race), 4 anaemia (11%, N>12 mg/dl) and 8 cases presented with no significant haematological alteration (22%), of which 3 were diagnosed by PCR.

CONCLUSIONS: All the cases occurred in African immigrants, mainly coming from Mali, except for one autochthonous case of someone working in Equatorial Guinea. The most common haematological alteration in our review was thrombopenia.

Haematology

Cod: 0678

BETA-2 MICROGLOBULIN AND RENAL FAILURE IN MULTIPLE MYELOMA

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BACKGROUND: Multiple myeloma (MM) is a cancer of plasma cells in the bone marrow.The abnormal proteins from the myeloma cells can cause kidney damage through a variety of mechanisms. Beta 2-microglobulin (B2M), a protein secreted by B-cells that correlates to myeloma cell mass (also indicates kidney damage). The present study aims to determine the level of creatinine, C-reactive protein (CRP), B2M, albumin, serum protein electrophoresis (SPE) and 25OH vitamin D (25-OH D) in serums of 20 patients with MM in the Republic of Kosovo.

METHODS: We analyzed samples from 20 patients with an average age of 60, which were diagnosed with MM at the Hematology Clinic of Prishtina in Kosovo. The control group was made up of 20 healthy individuals. Patients were divided into two groups: those that had renal failure (8 patients) and those that did not (12 patients). B2M and CRP was determined with immunoturbidimetry, other parameters by photometry,SPE by Minicap SEBIA, 25 (OH) D by ELFA.

RESULTS: There was a significant increase of B2M in patients with MM (11.4mg/ml) compared to the control group (1.78mg/ml). A significant increase of B2M ($p<0.001$) was noticed in the group that had renal failure (creatinine >200μmol/) compared to the group that did not (creatinine <200 μmol/L). BM2 indicate a positive correlation with the level of creatinine (+0.77). CRP in patients with MM is significantly different to the control group ($p<0.001$). CRP in patients with renal failure (76.8mg/l) is also very different to patients that do not have renal failure (8.6mg/l). Albumin is lower in patients with MM (34.1g/L) compared to the control group (41.7g/L), B2M levels are inversely correlated to serum albumin (-0.25). Gamma Globulin fraction shows a significant increase (31.5%) compared to the control group (13.4%), 25 (OH) D of patients with renal failure have lower levels of 20 ng/ml, however it does not differ significantly with patients that do not have renal failure ($p=0.3$).

CONCLUSIONS: Based on our results, we conclude that determining B2M highly correlates with renal failure in MM patients, thus making it one of powerful prognostic parameters. CRP may also be used to determine prognosis in patients with MM. The current study found no correlation between vitamin D status and MM activity.

Haematology

Cod: 0679

THE ROLE OF IGG SUBCLASS DISTRIBUTION AND HEVYLITE® MEASUREMENTS IN THE MONOCLONALITY OF IGG IN MULTIPLE MYELOMA OR MGUS

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BACKGROUND: Patients with multiple myeloma may not only present with elevated monoclonal IgG but also with polyclonal hypogammaglobulinemia. The role of the distribution of IgG subclasses in the monoclonality of IgG in multiple myeloma or MGUS has not been examined in detail and different methods have been applied in older publications with the most recent studies published more than two decades ago.

METHODS: In 109 patients (40 MM, 22 MGUS, 47 other underlying conditions) with distinct monoclonal IgG fraction as established by immunofixation, IgG subclasses (The Binding Site) were determined with the turbidimetric SPAPlus® Analyzer (The Binding Site). For further clarification of immune suppression, heavy and light chain specific determination of IgG was performed with Hevylite® (n=94).

RESULTS: In 24 patients, subclass distribution was within the reference range. Of the remaining 85 patients, IgG1 was elevated in 36.7% and deficient in 8.3%; IgG2 was elevated in 9.2% and deficient in 16.5%; IgG3 was elevated in 12.0% and deficient in 21.1%; IgG4 was elevated in 1.3% and deficient in 13.8%. While distribution among the various underlying conditions was comparable, MGUS patients had no IgG1 deficiency. In 11% a combination of IgG1 elevation and IgG2 deficiency was found. Of note, this was found in 22.5% of MM patients. The Hevylite ratio was determined in 94 patients and pathological ratios were found in 51.1%. Hevylite pair suppression was found in 35% of MGUS patients and 61.8% of MM patients. In 17 (89.5%) of the 19 MM patients with SK suppression, Hevylite pair suppression was found.

CONCLUSIONS: The combination of IgG1 elevation and simultaneous IgG2 deficiency was significant in our patient collective and markedly so in the MM patients. The role of IgG subclass distribution in patients with monoclonal IgG regarding prognosis and therapy has not been examined to date. Therefore, our study provides a first basis for new methods of analysis. The Hevylite results successfully mirrored the subclass distribution in the patients thus demonstrating that monoclonal elevation of one IgG subclass may lead to suppression of another IgG subclass. Suppression of the non-involved IgG chain was markedly pronounced in MM patients.

Haematology

Cod: 0680

POLYCLONAL AND MONOCLONAL ANTIBODY BASED FLC ASSAYS PROVIDE DISCREPANT INFORMATION FOR MONITORING RELAPSED MULTIPLE MYELOMA PATIENTS

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BACKGROUND: Guidelines for the assessment of serum free light chain (FLC) in multiple myeloma (MM) patients are based upon the Freelite® assays, which utilise polyclonal antisera. New assays calibrated to Freelite but utilising monoclonal antisera, NLatexFLC, have become available. Here we assess the performance of the new assays compared to Freelite as tools to monitor relapsed MM patients.

METHODS: Sequential sera from 42 relapsed MM patients (18IgGκ, 9IgGλ, 7IgAκ, 3IgAλ, 3 LC only, 2 bi-clonal; sample number, median(range): 7(2-16); follow-up: 192(7-827) days) treated with melphalan, dexamethasone and bortezomib were analysed retrospectively with Freelite (The Binding Site Group Ltd, UK) and NLatexFLC (Siemens, Germany) immunoassays. FLC measurable disease was defined as involved FLC (iFLC) levels >100mg/L with an abnormal FLC ratio. Percentage reductions in dFLC (iFLC–uninvolved FLC) were correlated between the assays using Pearson's linear regression. Good agreement was estimated at 95% limits of agreement <40% by Bland-Altman test. Normal reference range for FLCκ/λ ratio by Freelite: 0.26-1.65; by NLatexFLC: 0.31-1.56.

RESULTS: At baseline 40(95%) patients had an abnormal FLC ratio by Freelite (28κ: 73.68(1.94-1411.00), 12λ: 0.01(0.002-0.14)) compared to 38(91%) by NLatexFLC (25κ: 9.37(1.59-324.10), 13λ: 0.04(0.003-0.30)). 17 patients had measurable disease by both assays and 11 by neither; 1 patient was evaluable by NLatexFLC only; this patient had an abnormal FLC ratio (0.14) and involved FLC=58.56mg/L by Freelite. By contrast, 13 patients had measurable disease by Freelite only; all had an abnormal NLatexFLC ratio, 5 patients had low (<50mg/L) iFLC, and in 5 patients iFLC levels were within the normal range. There was poor agreement in response to treatment between the assays for 17 patients with measurable disease by both assays (Pearson: y=0.69x-0.14; R2=0.49; Bland-Altman 95% limits of agreement:-55% to 63%).

CONCLUSIONS: MM patient assessment using the Freelite assay has formed the basis of international guidelines. Assays calibrated to Freelite may be expected to have similar performance with respect to patient monitoring, however we were not able to show this in our population; further work is required to establish the utility of the new assays.

Haematology

Cod: 0681

A NEW VARIANT 12P13 REARRANGEMENT ASSOCIATED WITH EOSINOPHILIA IN A CASE OF REFRACTORY ANEMIA

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BACKGROUND: The most common chromosome rearrangement associated with eosinophilia in myelodysplastic/myeloproliferative syndrome (MDS/MPD) is t(5;12)(q33;p13). T(5;12) juxtaposes the TEL gene at 12p13 with the platelet derived growth factor receptor-beta (PDGFRb) gene at 5q33.This lead to formation of the fusion oncogene TEL/PDGFRb responsible for the malignant transformation. Cytogenetic variants of t(5;12) with eosinophilia are very rare in myeloid malignances . We describe a new variant 12p13 rearrangement in a case of refractory anaemia associated with eosinophilia. The patient, an 89-years male had proliferation of mature eosinophils in the peripheral blood (47%) and bone marrow (30%). During disease progression the eosinophilia was disappeared and elevation of monocytes was observed.

METHODS: Conventional cytogenetic studies were performed on bone marrow aspirate samples using standard Giemsa trypsin G-banding procedure. Karyotypes were recorded using the International System of Human Cytogenetic Nomenclature. Fluorescent in situ hybridization (FISH) were carried out with whole chromosome DNA probes for N°12 and N°7 and break-apart probe of TEL oncogene (12p13). FISH examinations were performed according to the manufacturer's instructions.

RESULTS: The cytogenetic analysis showed the following pathological karyotype: 46,XY,der(7)t(7;?)(q22;?)der(12)t(12:?)(p12.2;?)[6]/47,XY,idem, +8[6]/45,XY, idem, -11[2]/46,XY, idem, +8,-11[2]. FISH with break-apart probe of TEL oncogene demonstrated only one signal in karyotype. Combination of whole chromosome probes of N°7 and N°12 and break-apart probe of TEL demonstrated that the signal of TEL is located on normal homologue 12. The FISH examination indicated also that in derivative chromosome 12 additional material of unknown origin had replaced the segment 12p12.2->pter – der(12)add(12)(p12.2) and in derivative chromosome 7 small segment from 12p and additional material of unknown origin had replaced the segment 7q22->qter – der(7)t(7;12)(q22;p?) add(7)(q22).

CONCLUSIONS: Our result showed that t(7;12) is not balanced and TEL oncogene is deleted in the rearranged homologue 12. We supposed that in our particular case TEL oncogene is related to the pathogenesis as a tumor suppressor gene.

Haematology

Cod: 0682

COMPARISON OF THE EFFICACY OF SERUM C-REACTIVE PROTEIN, PROCALCITONIN, INTERLEUKIN-6 LEVELS AND NEW LEUKOCYTE PARAMETERS IN THE DIAGNOSIS OF NEONATAL SEPSIS

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BACKGROUND: Sepsis is an important cause of morbidity and mortality among newborn infants. Early and definitive diagnosis of neonatal sepsis is difficult because its signs and symptoms are non-specific; blood culture results may take longer than 48-72 hours and occasionally be false negative. Various studies have shown that there are some changes in the morphology of leukocytes which occurs during infections. These parameters, such as neutrophil and monocyte volume, conductivity, scattering and volume distribution width can be determined by current hematology analyzers. Aim of the study was to investigate and compare the efficacy of serum CRP, PC, IL-6 levels and parameters of mean neutrophil and monocyte volume, conductivity, scattering and volume distribution width for the diagnosis of neonatal sepsis.

METHODS: A total of 227 newborns (132 males, 95 females) were involved in the study. There were 116 cases in the sepsis group and 111 cases in the control group. Control group consisted of gestational age matched infants without sepsis. Venous blood samples were collected from infants at the time of diagnosis in sepsis group and complete blood count, peripheral blood smear, blood cultures, C-reactive protein (CRP), procalcitonin (PC) and interleukin-6 (IL-6) levels were measured. LH780 hematological analyzer (Beckman Coulter, Fullerton, CA, USA) was used to determine mean neutrophil and monocyte volume (MNV, MMV), conductivity (MNC, MMC), scattering (MNS, MMS) and, volume distribution width (NDW, MDW)

RESULTS: MNV, NDW, MMV and, MDW values were higher in septic patients than control group ($p<0.05$). MNS values was lower in the patients with sepsis ($p=0.002$). Predictive values of MNV, NDW, MMV and, MDW in the diagnosis of neonatal sepsis were found as lower than of CRP, PCT and IL-6.

CONCLUSIONS: Although the predictive values of parameters of mean neutrophil and monocyte volume, conductivity, scattering and volume distribution width in the diagnosis of neonatal sepsis were lower than of CRP, PCT and IL-6, these new parameters may be useful in differential diagnosis of newborn sepsis, along with other screening parameters. Among these parameters, especially MNV seems the most useful parameter with the highest specificity.

Haematology

Cod: 0683

NEW APPROACH OF THE HEMATOLOGICAL PARAMETERS AND RED CELL INDICES IN THE DIFFERENTIATION BETWEEN SICKLE-CELL DISEASE AND SICKLE-CELL THALASSEMIA AND APPLICATION TO BETA-THALASSEMIA TRAIT AND IRON DEFICIENCY ANEMIA

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BACKGROUND: In Tunisia, thalassemia and sickle cell disease represent the most prevalent monogenic hemoglobin disorders with 2.21% and 1.89% of carriers, respectively. This study aims to evaluate the diagnosis reliability of a series of red blood cell indices and parameters in differentiation of beta-thalassemia trait (! -TT) from iron deficiency anemia (IDA) and between homozygous sickle cell disease (SS) and sickle cell-thalassemia (ST).

METHODS: The study covered 384 patients divided into three groups. The first one is composed of 145 control group, the second consists of 57 ! -TT and 52 IDA subjects and the last one with 88 SS and 42 ST patients. We calculated sensitivity, specificity, positive-predictive values, negative-predictive values, percentage of correctly identified patients and Youden's index for each indice. We also established new cut-off values by receiver operating characteristic curves for each indice. An evaluation study was performed on another population composed of 106 ! -TT, 125 IDA, 31 SS and 17 ST patients.

RESULTS: Srivastava Index, mean corpuscular hemoglobin, red blood cell, Mentzer Index (MI) and mean corpuscular hemoglobin concentration show the highest reliability in discriminating ! -TT from IDA with new cut-offs slightly different from those described in literature. Ehsani Index, mean corpuscular volume, MI, Shine and Lal Index and Sirdah Index are the most powerful in the differentiation between SS and ST.

CONCLUSIONS: The effectiveness and the simplicity of calculation of these indices make them acceptable and easy to use for differential diagnosis.

Haematology

Cod: 0684

A LACK OF VALUE IN REFLEX TESTING OF HYPOGAMMAGLOBULINEMIA BY SERUM PROTEIN ELECTROPHORESIS

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BACKGROUND: Reduced gamma fraction or hypogammaglobulinemia in serum protein electrophoresis (SPE), a technique that detects and quantifies monoclonal immunoglobulins (M-protein), is associated with immune-suppression and -deficiency states. Hypogammaglobulinemia in the absence of a detectable M-spike on SPE has often been a cause for further investigation e.g. by immunofixation electrophoresis (IFE). While the optimal cutoff for hypogammaglobulinemia has been proposed, the overall improvement, if any, in M-protein detection with this approach has never been characterized. The objectives of this study are to (1) compare the sensitivity and positive rates for M-protein among SPE-negative hypo-, normo- and hyper-gammaglobulinemic patients, and (2) determine if reflex testing by IFE on hypogammaglobulinemia is justified.

METHODS: We carried out a retrospective study at the Sunnybrook Health Sciences Centre on all SPE-negative patients i.e. no visible M-spike from January 2010 to June 2013. Only cases with IFE performed within three weeks of an SPE were included. SPE and IFE were performed on the Sebia Capillarys™ 2 and Hydrasys™ systems respectively.

RESULTS: Of the 3298 SPE negative cases, 369 (11.2%) were IFE positive, with intact immunoglobulins (IgG, IgA or IgM) making up the majority (93.3%) of these positive cases. The M-protein positive rates for the hyper- (>17 g/L), normo- (7-17 g/L) and hypo-gammaglobulinemic (≤6 g/L) groups were 15.2%, 10.2% and 15.6% respectively. The sensitivity, on the other hand, decreased dramatically from 90% with the gammaglobulin cutoff at ≤17 g/L to 15% and 6% at cutoffs of ≤6 g/L and ≤4 g/L respectively.

CONCLUSIONS: The low M-protein positive rate (16%) and poor sensitivity (15%) in hypogammaglobulinemia (≤6 g/L) seriously challenged the value and practice of reflex testing based on reduced gammaglobulin concentrations on SPE as a large number of M-protein positive cases (85%) would still have been missed. The false negative rate of 11% by SPE alone has also underscored its limitation as the first line diagnostic test for monoclonal gammopathy.

Haematology

Cod: 0685

A QUICK SCREENING PANEL FOR DETECTION OF MONOCLONAL GAMMOPATHIES IN PATIENTS WITH INCIDENTAL CLINICAL FINDING

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BACKGROUND: The combination of quantification of serum free light chains (sFLC) and serum protein electrophoresis (SPE) enables sensitive quantification of monoclonal component in the study of monoclonal gammopathies. This protocol (SPE+sFLC) can help us to detect a monoclonal gammopathy in patients with incidental clinical findings without diagnosis at Emergency Service of the Hospital.

METHODS: we studied three patients admitted to Emergency Service where we found incidental clinical finding characteristic of multiple myeloma (anemia, hyperproteinemia, intense bone pain). Sera of the three patients were sent to the Laboratory of Immunology for the screening of a monoclonal gammopathy. SPE were performed on CAPILLARYS 2 (Sebia) and the sFLC were measured by FREELITE (The Binding Site) turbidimetric assay.

RESULTS:
Case 1 (Man, 68 years)
Clinical finding: macrocytic anemia (9.0 g/dl hemoglobin), rouleaux formation of erythrocytes, discrete pancytopenia.
Protocol SPE+sFLC: weak peak in SPE (0.10 g/dl), sFLC ratio very altered (free kappa=14450 mg/l, free lambda=4.9 mg/l, ratio=2949) and immunoparesis.
Diagnosis: Light Chain Kappa Multiple Myeloma Stage 3 ISS
Case 2 (Woman, 65 years)
Clinical finding: hyperproteinemia (12 g/dl), hyperviscosity and thrombocytopenia.
Protocol SPE+sFLC: large peak (3.28 g/dl), altered sFLC ratio (free kappa=617 mg/l, free lambda=11.1 mg/l, ratio=55.59)
Diagnosis: Multiple Myeloma IgG Kappa Stage 2 ISS
Case 3 (Woman, 64 years)
Clinical finding: intense back pain
Protocol SPE+sFLC: large peak (3.22 g/dl), altered sFLC ratio (free kappa=3.15 mg/l, free lambda=102 mg/l, ratio=0.031)
Diagnosis: Multiple Myeloma IgA Lambda Stage 3 ISS

CONCLUSIONS: In the context of clinical symptoms (bone pain, pathologic fractures, anemia, hyperproteinemia, hypercalcemia) that are alerts to suspect multiple myeloma it is advisable to apply the protocol (SPE+sFLC) for the screening of monoclonal gammopathies in patients without obvious clinical diagnosis. The combination of SPE and sFLC yields a fast and highly sensitivity approach in the screening of monoclonal gammopathies.

Haematology

Cod: 0686

ADIPOCYTOKINE LEVELS IN DIFFERENT TYPES OF BETA-THALASSEMIAS FOR CHILDREN

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BACKGROUND: Beta-thalassaemia is an inherited blood disorders. Beta-thalassaemia results from the impaired production of beta-globin chains, leading to a relative excess of alpha-globin chains. Clinical severity distinguishes this disease into three main subtypes; β-thalassemia major, β-thalassemia intermedia and β-thalassemia minor, the former two being clinically more significant. Inflammatory processes may play an important role in some of the complications of thalassemia. Adipose tissue is one of the most important endocrine and secretory organ that release adipocytokines like adiponectin, resistin and visfatin. The aim of our study was to analyze adipocytokines, which are adiponectin, resistin and visfatin, levels in different types of beta thalassemia patients and determine any possible correlations with disease severity.

METHODS: We recruited 29 patients who are transfusion-dependent beta thalassaemia-major patients, 17 patients with beta thalassemia intermedia, 30 beta-thalassemia minor patients. The control group consisted of 30 healthy children. Anthropometric measurements, complete blood count, biochemical parameters, serum levels of adiponectin, resistin, visfatin were performed for all subjects.

RESULTS: Resistin and visfatin levels were significantly higher in beta thalassemia minor patients than in controls. Adiponectin, resistin and visfatin levels were significantly higher in both beta thalassemia intermedia and major patients than in controls. The levels of adiponectin, resistin and visfatin were significantly higher in both beta thalassemia intermedia and major patients than in beta thalassemia minor patients. There was no significant difference between beta thalassemia intermedia and beta thalassemia major patients for adipocytokines levels.

CONCLUSIONS: We speculate that these adipocytokines may play an important role in the development of the complications of beta-thalassaemias.

Haematology

Cod: 0687

DO MIR-29A AND MIR-96 HAVE ANY EFFECT UP-REGULATION ON HB F LEVELS?

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BACKGROUND: MicroRNAs (miRNAs) are short non-coding RNAs of 19 to 25 nucleotides in length that regulate gene expression post-transcriptionally. Approximately 1,800 human miRNA sequences have been identified so far and they have used as diagnosis and prognosis biomarker in many human diseases. miRNAs can also regulate hematopoeitic differentiation. Our study focused on expression levels of miRNA that were taken from cases with normal (Hb AA) and cases with sickle cell anemia (Hb SS) which had high and normal HbF levels determined by RT-PCR method.

METHODS: We collected 3ml of whole blood in tube with K3EDTA from 84 cases. We analyzed hematological data and separated leukocytes from these cases. miRNAs isolated from these leukocytes then miR-29a and miR-96's gene expression levels were determined by RT-PCR.

RESULTS: In this study Hb SS with Hb AA cases for differential miRNA expression level by RT-PCR. Mann-Whitney U test were used to compare miRNA expression levels between two group. We considered statistically significant when the $p<0.05$ in these groups. miR-29a was overexpressed in Hb AA cases, while high Hb F levels of Hb SS cases and normal Hb F levels of Hb SS cases were down expressed. According to our study, miR-96 has not found in leukocytes cell of two groups.

CONCLUSIONS: Hb SS is the most common hereditary blood diseases in the World. It observed in some case with Hb SS that the level of Hb F has increased and the clinical course of these cases found to be milder, accordingly. In our study have showed that miR 29a has no role in the rise of the Hb F level and miR-96 has not been in leukocytes cells.

Haematology

Cod: 0688

COMPARISON OF THE PERFORMANCE OF THE NEWLY DEVELOPED IGA HEAVY CHAIN/ LIGHT CHAIN IMMUNOASSAYS WITH SERUM PROTEIN ELECTROPHORESIS AND NEPHELOMETRIC TOTAL IGA MEASUREMENTS FOR MONITORING IGA MULTIPLE MYELOMA PATIENTS

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BACKGROUND: Both serum protein electrophoresis (SPEP) and total immunoglobulin (tIg) measurements are recommended for quantification of monoclonal Ig (M-Ig). However, SPEP may be inaccurate at low concentrations and when M-Ig co-migrates with other serum proteins. By contrast, tIgA is an accurate method but is unable to distinguish between monoclonal and polyclonal Ig. Newly developed Heavy/ light chain immunoassays may provide an alternative method of quantifying M-Ig concentrations. Here we compare the performance of these assays with traditional methods for monitoring MM patients.

METHODS: HLC IgAκ and IgAλ were quantified in 61 MM (37 IgAκ, 24 IgAλ) patient sera. The results were compared to the published normal ranges (IgAκ (g/L): 0.48-2.82, IgAλ (g/L): 0.36-1.98, IgAκ/IgAλ: 0.80-2.04), historic SPEP, immunofixation and tIgA concentrations. Weighted Kappa and Pearson correlation were used to analyse results.

RESULTS: At presentation all 61 patients had an abnormal HLCr and involved HLC (iHLC) concentrations (median (range) IgAκ: ratio 233 (10-6226), iHLC 34 g/L (6-79); IgAλ: ratio 0.0119 (0.0003-0.1181), iHLC 29 g/L (6-72.0)). By contrast, M-IgA concentrations were measurable by SPE in just 66% (40/61) patients and the remaining 33% (21/61) were measured by tIgA. iHLC showed a good agreement with SPE ($y=0.88x+0.88$, $R^2=0.87$) and tIgA ($y=0.87x-0.68$, $R^2=0.90$) measurements at presentation and during follow up. iHLC changes also showed a good agreement with changes in SPE ($y=1.31x+0.25$, $R^2=0.87$) and tIgA ($y= 0.94x-0.03$, $R^2=0.90$). Weighted Kappa analysis showed a near perfect agreement between the responses assigned by either changes in iHLC and SPE/tIgA (89% agreement, Weighted Kappa (95% CI): 0.92 (0.84-1.00)) or changes in HLCr and SPE/tIgA (73% agreement, Weighted Kappa (95% CI) 0.86 (0.81-0.91)).

CONCLUSIONS: The recommended methods for monitoring M-Ig concentrations are limited to sensitivity and technical issues. The HLC immunoassays provide an alternative method of quantifying M-Ig in patients with MM.

Haematology

Cod: 0689

FREQUENCY OF IGG, IGA AND KAPPA AND LAMBDA LIGHT CHAINS IN MULTIPLE MYELOMA PATIENTS IN KOSOVO

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BACKGROUND: The aim of our study was to determine the frequency of IgG, IgA, kappa and lambda light chains in patients diagnosed with multiple myeloma. The disease affects plasma cells that produce immunoglobulin. Multiple myeloma is characterized by excessive numbers of abnormal plasma cells in the bone marrow and overproduction of intact monoclonal immunoglobulin (IgG, IgA, IgD, or IgE) or Bence-Jones protein (free monoclonal light chains).The most common type of heavy chain produced in myeloma is IgG, followed by IgA and then IgD. Occasionally, patients with myeloma produce incomplete immunoglobulin, containing only the light chain portion of the immunoglobulin.

METHODS: In the study, we analyzed serum samples from 49 individuals, 29 patients (19 males and 10 females with an average age of 63) that were clinically diagnosed with multiple myeloma and 20 healthy individuals (12 males and 8 females). The samples were tested for IgG, IgA, kappa and lambda by immunoturbidimetric assay (Cobas Integra 400/800).

RESULTS: Based on our findings, 48% of the patients had high levels of IgG (24% of them had high levels of kappa light chain and 24% had high lambda levels), 24% had high levels of IgA (14% of them also had high levels of kappa and 10% high levels of lambda), and 28% had high levels of light chains, kappa myeloma (17%) and lambda myeloma 11%). Serum concentration of IgG, IgA, kappa and lambda light chain in multiple myeloma patients were significantly higher than in the control group (25.5 g/L versus 11.3g/L, $p<0.001$; 9.59g/L versus 2.24, $p<0.001$; 5.34g/L versus 2.09 g/L, $p<0.001$; 4.0g/L versus 1.25g/L, $p<0.001$).

CONCLUSIONS: Referring to our study we can conclude that the most frequent type of immunoglobulin in tested multiple myeloma patients is IgG, followed by IgA and then light chain types, kappa and lambda.

Haematology

Cod: 0690

DISCREPANCIES BETWEEN DIFFERENT QUANTITATIVE ALBUMIN DETERMINATION METHODS IN PATIENTS WITH MONOCLONAL GAMMAPATHIES

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BACKGROUND: Spectacular advances in diagnostics and therapy of hematological diseases were connected with possibilities of precise monitoring of patients status. Monitoring of disease evolution and effect of the therapy are possible by accurate and precise laboratory data. Automation and technological progress in clinical laboratory has led to the situation that results obtained by different methods are comparable and precision of the numerical results is in the range of 2-5%. We investigated coherence of quantitative determinations of serum albumin assayed by colorimetric BCG method and electrophoretic procedure.

METHODS: Serum albumin (bromocresol green) and total protein (biuret) was quantitated by colorimetric technique using on Architect system. SPE electrophoresis on agarose gels were performed using Sebia Hyrys/Hydrasys system. Capillary electrophoresis was performed on Prince CE. Quantitative immunoglobulin determinations were performed on Simens BNII nefelometer.

RESULTS: As serum albumin assayed by colorimetric method end gel electrophoresis are in good agreement in normal sera. In patients with intensive monoclonal band results of this two laboratory tests are biased by considerable errors affecting possible clinical interpretation. Albumin concentrations were overestimated by 7g/l (~20%). This errors are immanent properties of the methodology. In case of quantitative determination of protein concentration based on densitometric measurements of electrophoretic separation, dye saturation effect of the gel and nonlinearity of the light transmission limits its accuracy at higher monoclonal immunoglobulin concentrations far exceeding values observed in any other pathology. Inaccurate gamma region quantitation affects quantitative albumin and monoclonal component level calculations. Validated capillary electrophoresis may correct this problem.

CONCLUSIONS: The results of the quantitative albumin, and monoclonal immunoglobulin assays in patients with high protein concentration and spectacular gammapathies should be discussed in good dialogue between biologist and clinician, and in combination with other laboratory tests as mechanical interpretation of the numerical data may result in errors affecting clinical treatment.

Haematology

Cod: 0691

COMPARISON OF TWO IMMUNOASSAYS FOR FREE LIGHT CHAIN KAPPA AND LAMBDA: ESTABLISHED (FREELITE) AND INCOMING (SIEMENS)

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BACKGROUND: International and national guidelines recommended serum free light kappa and lambda chains analysis in multiple myeloma and related disorders. These recommendations are based upon data generated using polyclonal Freelite assay (The Binding Site, UK). Recent advances in biotechnology have generated possibilities to investigate concentration of free kappa and lambda chains in serum and urine by measurement with new monoclonal antibody based immunoassay. The aim of this study was to compare the new N Latex FLC assay (Siemens, Germany) with Freelite ™ FLC assay.

METHODS: We analysed 125 samples of patients from whom 86 were diagnosed with multiple myeloma, 11 with lymphocytic neoplasms, 6 with other haematological disorders and the remaining 22 admitted for consultation of haematologist by measuring κ and λ FLC serum concentrations levels. Serum κ and λ concentrations levels were measured by nephelometry on a Siemens BN™II Analyser using two immunoassays: Freelite™ and N-Latex FLC κ and λ. Method Validator (www.method-validator.software.informer.com) software was used for statistical data analysis.

RESULTS: Concentrations of free light κ and λ chains by the Freelite™ ranged from 0.32 to 480.0 mg/L (mean value 35 mg/L) and from <0.05 to 368.0 mg/L (mean value 31.2 mg/L) respectively. Concentrations of free light κ and λ chains assayed by the N-Latex FLC ranged from 2.57 to 593.0 mg/L (mean value 34.3 mg/L) and from 1.0 to 305.0 mg/L (mean value 34.3 mg/L) respectively. Passing-Bablok regression analysis was used for the methods comparison. A relationship between both methods was found to be controversial. We found good agreement for free light κ (intercept: 0.699 with 95% confidence intervals [-0.913 to 1.960], slope: 1.041 [0.940 to 1.170], R2=0.92), moderate – for free light λ chains (intercept: 0.337 [-2.864 to 2.500], slope: 1.205 [1.000 to 1.508], R2=0.78), while for free κ/λratio – poor agreement (intercept: 0.180 [0.064 to 0.309], slope: 0.684 [0.569 to 0.788], R2=0.64).

CONCLUSIONS: Our findings indicate acceptable agreement between both Freelite™ and N-Latex FLCκ and λ assays but indicate systematic and proportional differences between κ/λ ratio.

Haematology

Cod: 0692

EVALUATION OF HYPERFERRITINEMIA IN A 5306-CASE SAMPLE IN BRAZIL: MEDIANS FOUND WERE CLINICALLY SIGNIFICANT

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BACKGROUND: Hyperferritinemia findings are common in clinical practice and present a diagnostic challenge to physicians due to the phenotypical and genotypical diversity that is involved in the buildup of this protein in the body. Even though there are many studies in the scientific literature about ferritin and its biological roles, there is not much about its elevated levels, especially not focusing on a prospective relation between high levels of ferritin and how severe tissue or body injury is.

METHODS: This study aimed at identifying the median levels of hyperferritinemia found in a sample of five thousand three hundred and six cases in the city of Sao Paulo which were obtained in random fashion and whose etiology is unknown. Subjects were patients of DASA Laboratory and samples were collected between September 2008 and February 2009. They were also sorted according to age group and sex. Serum ferritin was measured through chemiluminescence.

RESULTS: There was a higher incidence of hyperferritinemia in male individuals and in the age group that ranges from 20 to 59 years old. Median male hyperferritinemia from 0 to 9 years old was 1227.86 ng/mL; from 10 to 19 years old: 1251.90 ng/mL and from 20 to 59 years old, 616.17 ng/mL. For individuals 60 years old and over, 755.10 ng/mL. For female subjects, from 0 to 9 years old it was 737.92 ng/mL; from 10 to 19 years old: 1082.52 ng/mL; from 20 to 59 years old, 728.87 ng/mL and for individuals 60 years old and over, 669.72 ng/mL.

CONCLUSIONS: although great amplitude and variance were found in this study, there is clinical significance in median hyperferritinemia levels for both male and female patients. The levels ranged from 616.16 ng/mL to 1251.90 ng/mL, which calls for therapeutic intervention since iron overload poses a risk to vital organs. With further study it might be possible to stratify hyperferritinemia levels and create a new approach on the subject.
Keywords: Hyperferritinemia; ferritin; hemochromatosis; iron overload

Haematology

Cod: 0693

IMMUNOPHENOTYPIC AND CYTOMORPHOLOGICAL METHODS IN MULTIPLE MYELOMA

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BACKGROUND: Historically, that morphological study of the bone marrow is the standard diagnostic procedure for the diagnosis of multiple myeloma (MM). However, the differential diagnosis of paraproteinemia often similar clinical and laboratory data observed in many disease processes, and it is often difficult to diagnose timely. Multiparameter flow cytometry (MFC) immunophenotyping should become mandatory in the clinical management of hematological malignancies, in particular MM, both for diagnostic and monitoring purposes.

METHODS: We examined 212 patients with suspected MM. Examination conducted cytomorphological and immunophenotypic methods. MM was diagnosed in 62 patients (42 of them - women, 20 - men) with a median age of 63 years (range, 47-87 years). MFC immunophenotyping was performed using monoclonal antibodies against CD56, CD19, CD138, CD45 and IgG, IgA. Monoclonality was confirmed by immunoglobulin light chain analysis (κ, λ).

RESULTS: Cytomorphological bone marrow examination was diagnosed MM only in 20 cases (32.3%): it was detected more than 10% plasma (myeloma) cells in the bone marrow punctates. In other cases with only a mild plasmacytosis (<10% plasma cells), the diagnosis MM would be missed. Flow cytometry was confirm a diagnosis of MM in 62 cases (100.0%) in contrast to the simple morphologic analysis of the bone marrow. Immunophenotyping stem cells showed: in differential counts, plasma cells in bone marrow accounted for 0.5-52.1% of the total nucleated cell count. The positive expression rates of CD56, CD19, CD138, CD45 and immunoglobulin light chain κ, λ and IgG, IgA in neoplastic myeloma cells were 82.3%, 1.6%, 100.0%, 30.6%, 54.8%, 45.2%, 64.5% and 55.8% respectively.

CONCLUSIONS: Using flow cytometry, allows not only more accurate than morphological criteria to estimate the number of plasma cells in the bone marrow and peripheral blood, but also to characterize the degree of maturity (differentiation) and proliferation.

Haematology

Cod: 0694

FREQUENCY OF MUTATIONS IN THE HEMOCHROMATOSIS BRAZILIAN ADULT POPULATION WITH HYPERFERRITINEMIA: THE MOST PREVALENT MUTATION IN HETEROZYGOUS FEMALES WAS H63D (27%) C282Y MUTATION AND HETEROZYGOUS MALES (28%), IN 1038 CASES EVALUATED

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BACKGROUND: Hemochromatosis comprises a group of inherited disorders resulting from mutations of genes involved in regulating iron metabolism. The most common form among Caucasian populations of northern European origin is related to mutations in the hemochromatosis (HFE) gene, and 80% to 90% of Caucasian patients diagnosed with hemochromatosis in the United States are homozygous for the HFE C282Y. Another common mutation of HFE, H63D rarely is a cause of iron overload in the homozygous state or in the compound heterozygous state with C282Y .

METHODS: This study Aimed at Identifying which mutations of the hemochromatosis gene found in a population of 1038 adults in São Paulo - Brazil more frequently, who had high levels of serum ferritin.

RESULTS: The cases of hyperferritinemia match 4.7 male: 1 female on a sample of 1038 Brazilian adults with increased serum ferritin. The most prevalent mutation in females was heterozygous H63D mutation (27% of cases) and in men heterozygous C282Y mutation was the most frequently observed (28% of cases).

CONCLUSIONS: The frequency of HFE gene mutations in ambulatory patients with iron overload was 49.7% (516/1038) in general.

Haematology

Cod: 0695

IRON DEFICIENT ANEMIA AND IRON STATUS IN HEALTHY WOMEN FROM RURAL AND PERIURBAN AREA IN ALBANIA

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BACKGROUND: Iron deficiency and iron deficient anemia are recognized as a major public health problem throughout the world, especially in women and young children. The aim of this study was to evaluate the prevalence of iron deficiency with or without anemia in healthy women from high prevalence regions in Albania.

METHODS: 418 healthy women, aged 15-49 from rural area of Kukes and Shkoder and periurban area of Tirana, were included in the study. We evaluated hemoglobin (Hb) and Hb indices, ferritin and soluble receptors of transferrin (sTfR) to detect anemia and iron deficiency status. The prevalence of iron depletion as corrected by CRP values excluding those who have CRP > 10 mg/L. Hb analysis was performed with an Abbot Cell Dyn 1800 automatic analyzer, whereas serum ferritin and CRP were carried out using Immulite 1000 system. sTfR were measured by ELISA using R&D system reagent.

RESULTS: Anemia prevalence (Hb<12g/dL) in this group was 17.5%. The highest rate is found in periurban area of Tirana. The prevalence of depleted iron stores (ferritin<15 μg/L) was 23.6% whereas the prevalence of iron deficiency (sTfR>28nmol/l) was 8.1%. 16% of non anemic women were also having depleted iron stores.

CONCLUSIONS: Our data confirm that anemia poses a public health problem in women from the three selected regions.

Haematology

Cod: 0696

STABILITY OF RED BLOOD CELL PARAMETERS AFTER THREE DAYS

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BACKGROUND: Recommendation for EDTA whole blood analysis is within 6h of collection. Analysis after extended storage for up to 24h is not advisable even though certain situations make prompt analysis impossible. The aim of this study was to evaluate stability of hemoglobin (HGB), mean cell volume (MCV) and red blood cell (RBC) count after the period of prolonged storage (72h) at 4°C.

METHODS: Samples of 102 hospital patients were analyzed for HGB, MCV and RBC count on ADVIA 2120i (Siemens, Dublin, Ireland) hematology analyzer. Blood was collected for routine laboratory analysis in BD Vacutainer tube with K3-EDTA anticoagulant. Analysis was performed upon arrival into laboratory after which samples were stored at 4°C for further analysis. Coefficient of variation (CV) of analytical quality control for HGB was 1,50%, for MCV 1,01% and for RBC 1,43%. Statistical analysis was performed using MedCalc statistical software (Mariakerke, Belgium).

RESULTS: Results of measurement were compared using paired t-test where mean difference (MD) and standard deviation of MD were used for the difference assessment. MD were compared with CV of daily analytical quality controls. HGB concentration expressed as mean±SD at 0 and 72h was 120±25 and 120±25 g/L, MD±SD=-0,6±1,5 (0.5%). MCV expressed as mean±SD at 0 and 72h was 92,7±9,7 and 95,0±10,1 fL, MD±SD=2,28±1,43 (2,4%). RBC count expressed as mean±SD at 0 and 72h was 4,03±0,89 and 4,04±0,89 x 1012/L, MD±SD=0,01±0,05 (0.25%).

CONCLUSIONS: Although mean difference between two measurements for MCV (2.4%) is higher than CV (1.01%), for HGB and RBC MD is lower than CV. The results imply that whole blood samples can be used for HGB and RBC analysis after prolonged storage (for example over the weekend) if necessary.

Haematology

Cod: 0697

EFFECTS OF IRON THERAPY ON PARAOXONASE AND ARYLESTERASE ACTIVITIES IN PATIENTS WITH IRON DEFICIENCY ANEMIA

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BACKGROUND: Iron deficiency is the most common form of anemia. PON 1 is an antioxidant enzyme, synthesized by the liver and transported along the plasma bound to HDL. This calcium-dependent esterase has three known activities, paraoxonase, arylesterase and diazoxonase. It has been reported that paraoxonase-1 deficiency is related to increased susceptibility to development of atherosclerosis and cardiovascular disease. In this study, we aimed to investigate the relationship between PON1 activities in iron deficiency anemia (IDA). We suggest that iron treatment of anaemia promotes significant changes in serum PON1 activity and has a beneficial effect on oxidative stress in patients with IDA.

METHODS: Fifty adults with IDA and forty healthy were enrolled in this study. All patients were evaluated at inclusion in the study and after treatment using clinical and laboratory assessments. Complete blood count, ferritin, iron, total iron-binding capacity and other biochemical parameters were determined. Serum paraoxonase and arylesterase activities were measured with a spectrophotometer by using commercially available kits.

RESULTS: Mean paraoxonase and arylesterase activities in iron deficiency anemia group were significantly lower than mean activities of control group (102.44±19.29 U/L and 163.33±13.68 U/L, respectively and 157.37±26.43 U/L and 256.11±24.62 U/L, respectively; p =0.0001 for both). Paraoxonase and arylesterase activities significantly increased after treatment in iron deficiency anemia (143.28±13.98 and 197.61±27.95 U/L, respectively, p=0.0001). Mean activities of after treatment with iron were significantly lower than mean activities of control group (p=0.002; p=0 .0001 respectively).

CONCLUSIONS: In our study, paraoxonase and arylesterase activities in patients with iron deficiency anemia significantly increased after treatment with iron, but these results were still lower than the results of control group. Patients with longstanding iron deficiency may have increased risk for atherosclerosis and cardiovascular disorders in the later life due to decreased paraoxonase and arylesterase activities.

Haematology

Cod: 0698

COMPARISON OF CAPILLARY ELECTROPHORESIS AND AGAROSE GEL ELECTROPHORESIS FOR IDENTIFICATION OF MONOCLONAL PARAPROTEINS

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BACKGROUND: Monoclonal gammopaties has clinical and diagnostic importance for plasma cell dyscrasias and lymphoproliferative disorders. Serum protein electrophoresis and immunoelectrophoresis are used to identify monoclonal gammopathies such as multiple myeloma, Waldenström macroglobulinemia and other plasma cell dyscrasias. Agarose gel electrophoresis (AGE) is the most commonly used clinical method however capillary zone electrophoresis (CZE) has emerged a new sensitive technique for the separation of gammopathies.

METHODS: We compared CZE with AGE for the identification and characterization of monoclonal immunoglobulins. AGE and immunofixation electrophoresis (IFE) were performed on Helena SAS-I according to the manufacturer's directions. CZE and immunosubtraction method was performed on V8 Helena. The evaluation was carried out on 38 samples.

RESULTS: 5 samples were evaluated normal and 29 samples were identified to have the same monoclonal immunoglobulins by CZE and AGE. For the 38 specimens, 32 (84.2%) vs 31 (81.5) were found to have a band, 6 (15.7%) vs 7 (18.4) had no band for CZE vs AGE, respectively. Two samples that contained monoclonal immunoglobulins following by CZE, was not evaluated as abnormal by AGE. These two samples were identified to have IgG-kappa and IgG-lambda biclonal gammopathy by CZE wheras they were identified as normal by AGE. One sample that contained monoclonal immunoglobulins by AGE, was not evaluated abnormal by CZE. This sample was identified IgG-kappa, IgG-kappa and free lambda triclonality by AGE wheras it was normal by CZE immunosubtraction method. One sample that identified to have IgG-kappa and IgG-lambda biclonal gammopathy by CZE, was identified to have IgG-lambda monoclonal gammopathy by AGE.

CONCLUSIONS: Diagnosis and management of patients with monoclonal gammapathies depend on accurate identification and characterization of monoclonal proteins. IFE by agarose gel and immunosubtraction by capillary electrophoresis can be used alternatively in detecting type of monoclonal gammapaties. Laboratories should confirm the result with the other method in case of any inconvinience.

Haematology

Cod: 0699

MOLECULAR AND CYTOGENETIC FEATURES OF B-CELL CLONES EXPANDED IN MULTICLONAL B-CELL CHRONIC LYMPHOPROLIFERATIVE DISORDERS

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BACKGROUND: B-cell chronic lymphoproliferative disorders (B-CLPD) usually show a monoclonal expansion of a (single) mature-appearing aberrant B-cell clone. However, patients diagnosed with composite lymphomas and other B-cell chronic lymphocytic leukemias (CLL) have been reported for decades. This phenomenon, considered as a rare event, might have been underestimated due to the need for sophisticated multidisciplinary approaches encompassing histopathology, cytomorphology, immunophenotypic and cytogenetic techniques and/or molecular analyses of purified cell populations. In fact, B-cell neoplasms consisting of two phenotypically distinct populations of clonally unrelated B-lymphocytes coexisting in the same patient have an estimated overall frequency among B-CLPD patients of around 5%. Recently, it has also been shown that up to 20% of population-based non-CLL and CLL-like low count monoclonal B-cell lymphocytosis (MBLlo) cases may also carry two different unrelated B-cell clones. Potentially, such coexisting clones have a greater probability of interaction with common immunological determinants.

METHODS: We comparatively analyzed the B-cell receptor (BCR) repertoire and the molecular profile, as well as the phenotypic, cytogenetic and hematological features of 228 CLL-like and non-CLL-like clones between multiclonal (n=85 clones from 41 cases) versus monoclonal (n=143 clones) B-CLPD.

RESULTS: The B-cell receptor of B-cell clones from multiclonal cases showed a slightly higher degree of HCDR3 homology than B-cell clones from monoclonal cases, in association with unique hematological (e.g. lower B-lymphocyte counts) and cytogenetic (e.g. lower frequency of cytogenetically altered clones) features usually related to earlier stages of the disease. Moreover, a subgroup of coexisting B-cell clones from individual multiclonal cases which were found to be phylogenetically related, showed unique molecular and cytogenetic features: they more frequently shared IGHV3 gene usage, shorter HCDR3 sequences with a greater proportion of IGHV mutations and del(13q14.3), than other unrelated B-cell clones.

CONCLUSIONS: These results would support the antigen-driven nature of such multiclonal B-cell expansions, with potential involvement of multiple antigens/epitopes.

Haematology

Cod: 0700

THE EVALUATION OF TWO AUTOMATED HEMATOLOGY SYSTEMS: CELL DYN 3700 AND MINDRAY BC6800

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BACKGROUND: The performance of the haematology analyzer is one of the basic components in the determination of the productivity and efficiency of a haematology laboratory. The aim of this study is to determine the most suitable and productive haematology analyzer for our laboratory with a side-by-side comparison of the Mindray BC-6800 and Abbott Cell Dyn 3700 which has been used in our laboratory.

METHODS: The study population comprised one hundred whole blood samples of adult subjects. All samples were selected randomly from the Istanbul Faculty of Medicine Central Biochemistry Laboratory workflow and studied within a 2 hours time on the BC-6800 and Cell Dyn 3700. The imprecision, linearity, carry-over and comparison studies were done to evaluate the method performance according to the International Council of Standardization in Haematology (ICSL).

RESULTS: The findings of precision studies (inter-assay and between-day) for hemoglobin, hematocrit, MCV, white and red blood cells and platelets for the Cell Dyn 3700 and Mindray BC-6800 were acceptable which were lower than 3%. The data of carry-over studies for high and low were less than 0.5% for both systems. The linearity studies showed good correlations between expected and obtained values for all haematological parameters. Correlation coefficients were between 0.992 and 0.998 for both instruments. When the method comparison were done by EP Evaluator between Cell Dyn 3700 and Mindray BC-6800, the WBC, RBC, platelet and Hgb levels closely correlated (R values higher than 0.99). The accuracy of WBC differentials were also evaluated between two system. The correlation coefficients of basophils was lower (R=0.53) and for eosinophils (R=0.93).

CONCLUSIONS: The Mindray BC-6800 is an highly accurate, precise, and high-speed analyzer depending our results and can be chosen for medium and high-volume laboratories.

Haematology

Cod: 0701

COMPARISON OF MONOCLONAL ANTIBODY-BASED NEPHELOMETRIC ASSAY AND POLYCLONAL ANTIBODY-BASED ASSAY FOR SERUM FREE LIGHT CHAIN MEASUREMENT

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BACKGROUND: Serum-free light-chain (sFLC) assay are important disease biomarkers in patients with plasma cell-proliferative disorders. The sFLC tests provide an independent measurement of kappa (κ) and lambda (λ) FLC, and the calculation of κ/λ sFLC ratio. Some immunoassay methods have been developed to measure sFLC; monoclonal and polyclonal antibodies. The purpose of this study was to compare two analytical methods: the well-established sheep-derived polyclonal antibody-based Freelite® and the new monoclonal antibody-based nephelometric assay.

METHODS: Method comparison was performed with 100 serum samples. sFLC levels were measured by Freelite® (The Binding Site Group Ltd, Birmingham, UK) assay and the nephelometric assay (N Latex FLC, Siemens) using the BNII nephelometer (Siemens Diagnostics, Germany). κ/λ sFLC ratio was calculated. Data were expressed as mean ± standard errors. Bland-Altman analysis was performed to calculate limits of agreement.

RESULTS: The analytical sensitivities of both methods were defined as 0.30 mg/L for Freelite® κ, 0.25 mg/L for Freelite® λ and 0.17 mg/L for N Latex FLC κ, 0.47 mg/L for N Latex FLC λ. The within-run and between-run CVs obtained with controls were under the 10 %. The comparison showed the following relationship between two methods for κ/λ sFLC ratio: N Latex FLC = 0.76.Freelite® + 0.29 (r=0.92, r2=0.85). The bias between Freelite® and N Latex FLC κ/ λ sFLC ratio assay was -0.16(95% CI= -0.51- 0.18).

CONCLUSIONS: The analytical performance of two assays appeared comparable, but there are some differences in measurement of concentrations between the methods. Differences between these two assays have to be presumed to be due to the different detection reagents used as well as the different affinities and specificities of the antibodies. The monoclonal antibody-based N Latex FLC assay is a reliable alternative for the Freelite® method to monitor sFLC. It provides a valid quantitative measurement of sFLC with comparable % CVs in quality-control as well as patient serum samples.

Haematology

Cod: 0702

VALIDATION OF STRINGENT COMPLETE RESPONSE IN PATIENTS WITH MULTIPLE MYELOMA AFTER THERAPY WITH NOVEL AGENTS

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BACKGROUND: normalization of serum free light chains (sFLC) ratio in patients with Multiple Myeloma (MM) achieving complete response (CR) may define a deeper response after therapy than obtained by the CR criteria. The stringent CR (sCR) requires normalization of sFLC ratio and absence of clonal plasma cells in bone marrow in addition to the criteria for CR (Negative immunofixation of serum and urine and disappearance of any soft tissue plasmacytomas, and <5% plasma cells in bone marrow). The aim of this preliminary study is to evaluate the impact of sCR in patients with newly diagnosed MM.

METHODS: twenty three patients with MM (10 IgG MM, 5 IgA MM, 2 IgD MM and 6 Bence Jones MM) achieving CR after therapy with Bortezomib/Dexametasone were included in this preliminary study. We studied Disease Free Survival (DFS or time after treatment where disease remains stable) as prognostic factor. DFS was estimated by Kaplan-Meier method and compared by log-rank tests. Cox proportional hazard analysis was performed for multivariate analysis. Serum free light chains were measured by turbidimetry (Freelite) in a SPA PLUS analyzer (The Binding Site Group Ltd, Birmingham, UK) and immunofixation were performed in a HYDRASYS (Sebia, FR) analyzer.

RESULTS: the median follow-up of the patients was 18 months (range 14-31 months). Eleven patients achieved CR and 12 patients achieved sCR. During the period of study there were 8 relapses, six in patients achieving CR and two in patients achieving sCR. The median DFS for patients achieving CR was 18 months and not reached for those achieving sCR. Patients achieving CR had a DFS rate of 24% compared with 75% for sCR (p=0.022). Results showed that achieving a sCR was an independent prognostic factor for survival (HR = 6.57; 95% CI, 1.09-39.80; vs CR; p = 0.039).

CONCLUSIONS: the presence of an altered sFLC ratio represents the existence of a persistent clonal population that is secreting very small amounts of monoclonal protein. Our results indicate that sCR represents a deeper response state compared with conventional CR. Analysis of sFLC ratio was able to identify the favorable group of patients and support the inclusion of sFLC ratio as part of the response criteria for MM.

Haematology

Cod: 0703

CUTANEOUS LESIONS: CAUSE OF CRYOGLOBULINEMIC SYNDROME SECONDARY TO MULTIPLE MYELOMA

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BACKGROUND: Multiple myeloma (MM) is characterized by the neoplastic proliferation of plasma cells producing a monoclonal immunoglobulin. Cryoglobulins (CG) consist of immunoglobulins (Ig) or a mixture of Ig and complement components which precipitate at low temperature. CG can be detected in 5 to 10 percent of MM cases. Type I cryoglobulinemia is typically related to an underlaying lymphoproliferative disease. This type of cryoglobulinemia accounts for approximately 5 to 25 percent of MM cases.

METHODS: A 78 years-old male patient was admitted to emergency admissions referred from a dermatology consultation due to constitutional syndrome and cutaneous lesions of the lower extremities, hands, nose and outer ear. Three months before he went to emergency admissions because of cutaneous rash.

RESULTS: The clinical analysis revealed hypercalcemia, anemia and increased serum protein concentration. About 20-25 percent of plasma cells, granular lymphocytes, rouleaux formation, pseudothrombocytopenia and some immature cells were seen in the peripheral smear. The patient was referred to the hematology department due to a suspected MM vs plasma cell leukemia and the clinical analysis was extended. A monoclonal single narrow peak in the gamma region in serum and urine was seen. An IgG kappa was identified by serum immunofixation. The cryoprecipitate was positive, with a cryocrit of 87,2 percent. The bone marrow aspirate and subsequent analysis by flow cytometry revealed a decrease in erythroid and granulopoietic series due to the high infiltration of plasma cells, with pathological phenotype. FISH analysis in bone marrow biopsy showed a p53 deletion on 12 percent of nucleus analyzed.

CONCLUSIONS: The peripheral smear together with the presence of a monoclonal protein in the serum and urine led to the MM diagnosis, in this case with type I cryoglobulinemia associated. The bone marrow analysis confirmed the diagnosis. p53 deletion is associated with a worse prognosis. The patient had a fatal outcome because of his bad overall condition. We present this case in order to recommend to do an extensive hematological study in patients with cutaneous lesions as a result of cold exposure, since it might be related to an underlaying lymphoproliferative disease.

Haematology

Cod: 0704

PERITONEAL EFFLUENT CELL COUNT WITH THE AUTOMATED HEMATOLOGY ANALYZER ABX PENTRA DX 120 IN PATIENTS AFTER HEART TRANSPLANTATION WITH PERITONEAL DIALYSIS AND PERITONITIS

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BACKGROUND: Peritonitis remains a leading complication of peritoneal dialysis (PD) and major cause of patients discontinuing PD and switching to hemodialysis. PD patients with peritonitis usually present with cloudy fluid and abdominal pain. This is confirmed by obtaining effluent cell count, differential, and culture. An effluent cell count with white blood cells (WBC) more than 100/mL (after a dwell time of at least 2 hours), with at least 50% polymorphonuclear neutrophilic cells, indicates the presence of inflammation. Up to now the microscopic counting and the differentiation of WBC in a effluent smear have been used as a reference. The aim of our study was to evaluate the correlation between the manual cell counting with an automated procedure in effluent in patients after heart transplantation and PD using a Pentra DX 120 analyzer.

METHODS: We presented a clinical case of a patient after cardiac transplantation with peritoneal dialysis who on the 10th postoperative day developed clinical signs of peritonitis with abdominal pain and slightly cloudy fluid. We examined 20 effluent samples.The samples were collected in standard Li Heparin sampling tubes to avoid coagulation and preserve cells. All samples were analyzed with a Pentra DX 120 hematology auto analyzer. Manual counting was performed with a Fuchs -Rosenthal chamber and cytocentrifuge preparations with May-Grunwald-Giemsa staining for the leukocyte differential on the microscope. All data are presented as mean and coefficient of correlation (r2) by using one-way analysis of variance (ANOVA).

RESULTS: Two weeks after administration of antibiotic therapy WBC decreased from 1371 to 73/ml, PNM from 986 to 2.9/ml , MN from 403 to 69.6 m/l and without clinical evidence of peritonitis. We found statistically significant correlation ($p<0.001$) between the manual and automated cell counting. Correlation coefficient for the WBC was calculated with $r = 0.995$ and for the polymorphonuclear (PMN) and mononuclear population (MN) was $r = 0.997$ and 0.989.

CONCLUSIONS: This is the first report for analysis of effluent of peritoneal dialysis with automated method. The study shows excellent correlation for the WBC, PNM and MN between the automatic and manual methods.

Haematology

Cod: 0705

RETICULOCYTE HEMOGLOBIN (CHR) AS EARLY MARKER OF IRON THERAPY EFFICACY IN HEMODIALYSIS PATIENTS

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BACKGROUND: Anemia is a common feature in hemodialysis (HD) patients. The planning an adequate human recombinant erythropoietin (rHuEPO) and intravenous iron supplementation treatment may improve their quality of life. Hemoglobin values >11 g/dL is a acceptable goal in HD patients. Half-life's reticulocytes in peripheral blood is 1-2 days. Accuracy of content of reticulocyte hemoglobin (CHr), during supplementation for iron-deficient patients, is assessed as an early indicator of iron sufficiency tool rather than conventional iron parameters as serum ferritin, transferrin saturation an soluble transferrin receptor levels. European guidelines for the treatment of anemia in HD patients indicate CHr >29 pg as desirable goal. The aim of this study is the assessment of iron supplementation efficacy in HD patients through CHr monitoring.

METHODS: We recruited 80 HD patients, 53 males and 27 females in rHuEPO and intravenous iron therapy. The peripheral blood samples is collected with K3EDTA anticoagulant and automatic analyzer ADVIA SIEMENS 2120i performed complete blood counts (CBC) and CHr measurement within 3 hours after collection. Hb and CHr results have assessed at basal time and during the first and the second week after iron supplementation.

RESULTS: 43 HD patients have Hb <11 g/dL. 17 HD patients (first group) have received iron supplementation (180 mg/week), whereas clinical evaluation have excluded 26 HD patients (control group). In first group, CHr average is improved after first week from 28 pg to 29 pg, without Hb significant changes. After second week, Hb average is improved from 9,8 to 10,6 g/dL while CHr until 29,5 pg. In the second group (control group), that haven't received iron supplementation, we highlighted early Chr decreased and at second week a slight Hb reduction.

CONCLUSIONS:
1. CHr confirms the role of early predictor efficacy in intravenous iron supplementation therapy in HD patients.
2. The early decrease CHr rather than Hb, suggests the need to define a individual CHr minimal decision level for iron supplementation.
3. Chr accuracy is a tool for HD patients monitoring, bringing to a better saving management in iron and rHuEPO therapy and improving their quality of life.
4. Points 2 and 3 will be the object of our further study

Haematology

Cod: 0706

PERFORMANCE EVALUATION OF A NEW STFR ASSAY FOR THERMO SCIENTIFIC KONELAB CLINICAL CHEMISTRY ANALYZER

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BACKGROUND: The serum-soluble transferrin receptor (sTfR) is a truncated form of intact receptor. sTfR is an index of tissue iron needs and therefore the measurement of sTfR concentration gives valuable information about the iron storage status even before development of anemia. The level of sTfR is within normal range in inflammatory states without co-existing ID and therefore it helps in the differentiation between iron deficiency anemia and other anemias caused by chronic diseases. Thermo Scientific™ Konelab™ clinical chemistry analyzers (20, 20XT, PRIME 30, PRIME 60) are random access, fully automated and proven clinical chemistry systems. Colorimetric, turbidimetric and ISE methods are applicable and the analyzers are capable of handling routine and stat requests.

METHODS: Konelab (20XT, PRIME 30, PRIME 60) sTfR method with system reagents, calibrators and controls is a particle enhanced immunoturbidimetric assay using latex particles coated with mouse monoclonal antibodies against human sTfR. Serum and Li-heparin can be used as sample types. The increase in absorbance caused by formation of immunocomplexes is recorded at 575 nm.

RESULTS: The assay measuring range is 0.3 – 8.0 mg/l extended with automatic dilution up to 40 mg/l. The repeatability (within-run precision) is from 2.03 to 3.66 % (CV) for samples with sTfR concentrations from 1.11 to 5.55 mg/l (N=80). The within device (total) precision is from 3.10 to 5.23 % (CV) for samples with sTfR concentrations from 1.11 to 5.55 mg/l (N=80). A method comparison study was performed using a commercially available particle enhanced immunoturbidimetric method as the reference. The Konelab method correlated well with the reference method. Linear regression was y = 1.0x - 0.04 and r = 0.959 (N=113).

CONCLUSIONS: The results demonstrate that sTfR can be analyzed accurately and easily using Thermo Scientific Konelab clinical chemistry analyzer.

Haematology

Cod: 0707

REQUEST OF ANEMIA LABORATORY TESTS IN PRIMARY CARE IN SPAIN

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BACKGROUND: To compare the inter-practice and inter-regional variability in anemia laboratory tests requested by General Practitioners (GPs) in Spain, according geographic and hospital characteristics, using appropriateness indicators, to try to ascertain the degree in requesting appropriateness.

METHODS: 76 laboratories covering 17679195 inhabitants from diverse regions across Spain filled out the number of cell blood count (CBC), serum ferritin, folate, iron, transferrin and vitamin B12 requested by GPs for the year 2012. Every patient seen in any primary care center, regardless of the reason for consultation, was included in the study. Each participating laboratory was required to provide organizational data. Two types of appropriateness indicators were calculated: every test requests per 1000 inhabitants and ratios of related tests requests (folate/vitamin B12, ferritin/CBC, ferritin+transferrin/CBC, transferrin/CBC and transferrin/ferritin). The indicators results obtained in different geographical areas, type of management and in three communities were compared.

RESULTS: No significant differences were obtained in test requesting by GPs in rural, urban or rural-urban locations except for CBC/1000 inhabitants that was higher in rural areas. The indicator was also higher in private management areas when compared to public. In total, 0.36 ferritin were requested for every CBC. And it becomes 0.50 if transferrin is added. In every laboratory folate/vitamin B12 indicator result was very high (0.95). Castilla-Leon ferritin, folate, iron, transferrin and vitamin B12 per 1000 inhabitants indicators results were the highest when compared to other communities. It also happened with ferritin/CBC and transferrin/ferritin indicators values.

CONCLUSIONS: Primary care ferritin and transferrin requests seemed to be too high when compared to CBC. In spite of the use of the vitamin B12 in primary care for other purposes different to anemia, folate and vitamin B12 were requested at a one to one ratio. The high variability observed difficult to explain by differences in patient case mix between regions and the high test request emphasizes the need to accomplish interventions to improve anemia tests appropriate use.

Haematology

Cod: 0708

CO-INHERITANCE OF HB ALESHA [β-67(E11)VAL-MET, GTG-ATG] CAUSED BY A DE NOVO MUTATION AND THE ALPHA212 PATCHWORK GENE IN A BRAZILIAN CHILD WITH A SEVERE HEMOLYTIC ANEMIA

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BACKGROUND: Hemoglobin (Hb) Alesha is a rare, unstable β-chain variant in which valine (Val) was replaced by methionine (Met) at position 67. The larger Met residue in the heme pocket causes the loss of nonpolar bonds between the β-chain and the heme group, resulting in high instability and severe hemolytic anemia. The α212 allele in turn consists of the α2 gene sequence except in two sites of the second intron where the normal sequence is replaced by specific α1 gene sequences. While in one of the sites there is a T-G nucleotide substitution at position 55, in the other there is a substitution of a single base (G) at position 119 by an octanucleotide (position 119-126, 5´-CTCGGCCC-3´).

METHODS: The patient was an 11-month-old white female of mixed ethnic origin (native Indians and Europeans) from Midwestern Brazil with severe hemolytic anemia, blood transfusion dependence and bone deformation since the age of six months. Both the parents were normal. Hb was analysed by electrophoresis at alkaline and acidic pHs, isoelectric focusing (IEF), cation-exchange high-performance liquid chromatography (HPLC) and reverse phase(RP)-HPLC. For molecular analyses, the α- and β-globin genes were sequenced directly after cloning of the α2 gene.

RESULTS: In alkaline pH and in IEF, Hb Alesha migrated to the position of Fetal Hb; in the HPLC it co-eluted with HbA and in RP-HPLC, an anomalous ß-chain co-eluted with the δ and ß chains. Sequencing of the ß gene revealed the mutation corresponding to Hb Alesha, in heterozygosis, confirmed by the complementary DNA strand. Familial analysis (mother, father, sister and maternal grandmother) failed to reveal the mutation, which was therefore considered a de novo mutation in the child. Sequencing of the α2 gene identified the α212 patchwork, also in heterozygosis, and showed that the father was a carrier of this patchwork.

CONCLUSIONS: This is the first case in Brazil in which either Hb Alesha or the α212 patchwork has been described. It highlights the importance of the ß67Val for Hb stability and the Hb investigation in multiethnic origin populations.
FINANCIAL SUPPORT: FAPESP/CNPq, Brazil.

Haematology

Cod: 0709

HEMATOLOGICAL PARAMETERS AMONG KIDNEY TRANSPLANT RECIPIENTS

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BACKGROUND: Anemia is common after renal transplantation and is frequently under-treated. Renal transplant recipients restarting dialysis have lower haemoglobin (Hgb) levels when compared with non-transplant chronic kidney disease (CKD) patients, which correlate with higher mortality.

METHODS: In our study, we examined kidney transplant recipients who were treated with immunosuppressive drugs. We followed haematological parameters such as Hb, Htc and renal function test such as blood urea and creatinine as well as creatinine clearance using analyses of variance.

RESULTS: Anemia was defined as Hb<120 g/L for male patients and Hb<110 g/L for female patients. We identified anemia in 26% of patients. Most transplant recipients have an average glomerular filtration rate < 60ml/min. The anemic patients had a significantly higher mean blood urea and serum creatinine levels and lower mean creatinine clearance level than the non-anemic patients. Among the immunosuppressant drugs, patients on tacrolimus had significantly lower Hb and Htc compared with patients receiving cyclosporine. The presence of a hemoglobin <120 g/L at 3 months after kidney transplantation is a major risk factor for persistent anemia at the end of the first posttransplant year. Anemia at 12 months after transplantation is independently associated with reduced patient survival.

CONCLUSIONS: Treatment of anemia using iron therapy has been hypothesized to decrease the cardiovascular mobidity and mortality in renal transplant recipients.

Haematology

Cod: 0710

PORPHYRIAS – A LABORATORY EXPERIENCE IN A TERITARY CARE HOSPITAL

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BACKGROUND: Porphyrias are rare cases, arising due to a deficiency of different enzymes in biosynthesis of Haem, a disorder with limited experience to both clinicians and laboratories. The recent past with better understanding of spectrum of clinical manifestations and diagnostic modalities has definitely seen a larger number of references and diagnosis. This laboratory's experience has been with acute intermittent Porphyria (AIP), the most common variety seen in India and hence testing for Porphobilinogen (PBG) and Aminolevulinic acid (ALA) were done.

METHODS: Over the past 10 years 300 requests for assessing Porphyrias were received in the age group of 16 – 60 years with male predominance and 50 in pediatric age group. The diagnostic approach was with the random urine qualitative analysis by the Watson Scharwtz methodology. The positive were subjected to quantitative estimation of PBG and ALA by the exchange resin chromatography (Biosystems).

RESULTS: 16 subjects beyond pubertal age and 2 in the pediatric age groups showed elevation in PBG and ALA. The clinical manifestation of a) neuro visceral pain and b) electrolyte imbalance were prominent, followed by c) altered sensorium, d) psychiatric manifestations and e) weakness of limbs. The severity of clinical manifestation correlated well with elevated PBG and ALA. 2 of the 16 with elevated ALA were in the differential diagnosis of lead poisoning. An elderly patient was an example of acquired Porphyria due to long standing medication with alternative medication. Clinical improvement with Glucose infusion and other therapeutic modalities were monitored in the laboratory. Remarkable reversal of laboratory and clinical improvement was seen in two young subjects treated with Hematin, not easily available in India and expensive.

CONCLUSIONS: Simple laboratory testing with clinical history is essential for identifying this disorder of Heam synthesis either inherited or acquired. Timely and appropriate medication relieves patients of the variety of clinical spectrum of the manifestations preventing acute stages of morbidity.

Haematology

Cod: 0711

IRON PARAMETERS, NON-TRANSFERRIN BOUND IRON AND HEPCIDIN LEVELS AT DIAGNOSIS IN MULTIPLE MYELOMA AND THEIR IMPORTANCE FOR PROGNOSIS

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BACKGROUND: Anemia of chronic disease is an iron metabolism disorder that occurs in 60-80% of untreated multiple myeloma patients. Hepcidin attaches ferroportin and decreases iron release from macrophages and enterocytes, so its role in anemia of chronic disease, especially being in a close relationship with inflammatory response, becomes a question. Non-transferrin bound iron (NTBI)-an iron form which occurs when transferrin's binding capacity is exceeded-is an unstable molecule that generates oxidative stress and hence could be a marker for the iron disorder in the multiple myeloma.

METHODS: We evaluated iron parameters (iron, total iron binding capacity (TIBC), and ferritin), NTBI and hepcidin at the time of diagnosis in 40 multiple myeloma patients at various stages, and compared with values obtained from 29 healthy adults with matched age and gender.

RESULTS: Mean serum iron and TIBC levels of the patients were lower (iron:62µg/dl vs. 95µg/dl,p=0.000; TIBC:235µg/dl vs. 304µg/dl,p<0.0001); mean serum ferritin and hepcidin levels were higher (ferritin:379.6ng/ml vs. 97.8ng/ml,p=0.0001; hepcidin:252.3ng/ml vs. 67.73ng/ml,p<0.0001) compared to the control group. Median serum NTBI levels ranged between 3-4 µg/dl for the patients and 2-3 µg/dl for the controls with a significant difference (p<0.05). When the patients were grouped according to the Durie-Salmon Staging System, only median serum TIBC levels showed a difference (p<0.05) between stages (IA:337µg/dl, IIA:181µg/dl, IIIA:240µg/dl, IIIB:194µg/dl). Grouping the patients according to the International Staging System revealed that all parameters, except median serum iron were different between stages (TIBC: I:282µg/dl, II:208µg/dl, III:195µg/dl,p<0.05; ferritin: I:80.8ng/ml, II:180.45ng/ml, III:521.1ng/ml,p<0.05; hepcidin: I:63.83ng/ml, II:310.38ng/ml, III:388.23ng/ml,p<0.001; NTBI: I:3µg/dl, II:3µg/dl, III:4µg/dl,p<0.05). While median serum TIBC levels decreased with stage, hepcidin and ferritin levels increased. Consequently, there was no sign for iron overload.

CONCLUSIONS: We propose that the stage of the anemia might have a connection with the disease stage and possibly with the prognosis of newly diagnosed multiple myeloma patients. Using hepcidin, in addition to standard iron parameters, may be useful.

Haematology

Cod: 0712

DOES FOLIC ACID LEVELS VARY DUE TO AGE IN TOKAT REGION?

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BACKGROUND: The aim of this retrospective study was to examine folic acid levels in Tokat region and define the reference intervals by the age groups. We used the data of the patients who applied to Gaziosmanpasa University Health Research and Application Center Polyclinics in the year 2013.

METHODS: The total number of tests included in the study was 5696. Input of the patients from gynecology clinics, because of the high incidence of folic acid supplementation by the pregnant women, emergency service, hemodialysis and the intensive care units were excluded. Repeated measurements were removed totally. Statistical analysis was performed using MedCalc Version 13.0 (trial) and IBM SPSS Statistics Version 20 software packages.

RESULTS: 197 subjects were 7-11 age and the mean folic acid value was 10.07 ± 2.6 ng/mL. 12-18 aged 336 individuals' values were 8 ± 2.4. Folic acid levels of the 18-64 aged group and the ones older then 65 years were 8.1 ± 2.9. When the results were compared according to gender male (2197) and female (3499) folic acid levels were 7.87 ± 3; 8.66 ± 3.1, respectively. Both results were statistically significant.

CONCLUSIONS: Folic acid is a water soluble vitamin and is related to the dietary variants. It plays important roles in fetal growth, also in childhood and in adults. The levels of this vitamin can vary according to the regions and their dietary behaviours. So such kind of studies can help to determine real reference intervals of that region and individuals can be directed more healthy to the folic acid supplementation.

Haematology

Cod: 0713

TRICLONAL GAMMOPATHY IN A PATIENT WITH RELAPS MULTIPLE MYELOMA

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BACKGROUND: Multiple myeloma is a malignant disorder characterized by proliferation of single clone of plasma cells derived fromB-cells in the bone marrow. The M-protein is a tumor marker specific for monoclonal gammopathies because it reflects the clonal proliferation of immunoglobulin. The occurrence of two or more monoclonal (M) components in the serum of a same patient is very unusual. Most retrospective studies show that the incidence of biclonal gammopathies ranges between 1% and 2.5% of all gammopathies. Triclonalgammopathies are much rarer, and their incidence is unknown. They were associated with immunoproliferativedisorders, hematologic malignancies, and other diseasesor were of undetermined significance.

METHODS and RESULTS: Seventy-one years old male patient, not suitable for bone marrow transplantation, was diagnosed as IgA- lambda type multiple myeloma. Partial remission was obtained by melphalan (10 mg/ m^2/ day for 4 days) and prednisolon (100 mg/ m^2/ day for 4 days) combination after 6 cycle. After 3 years patients was admitted to hospital because of relapse, and biclonal IgA and biclonal lambda M- band was detected in serum. Then combination of bortezomib (1.3 mg/ m^2/ day for 4 days), adriablastina (9 mg/ m^2/ day for 4 days), and dexamethazone (40 mg/day for 4 days) was administered for 6 cycles, and complete remission was obtained. Then patients was given thalidomine (200 mg/day) for maintanence therapy. After 10 months of maintenance therapy IgA- lambda M- band was detected in serum immunefixation electrophoresis. Since patients was asymptomatic, maintanence was continued. At the 17th months of maintanence serum immunefixation electrophoresis revealed triconal IgG, monoclonal IgA, biclonal kappa and biclonal lambda M band. M- band was not detected in urine immunefixation electrophoresis and patient was diagnosed as relapse.

CONCLUSIONS: A review of the literature indicates that the presence of two or more M-components is uncommon. Triclonal gammopathies are very rare, and relatively little is known about their clinical significance. The conventional technique serum electrophoresis is still widely used for the demonstration of M-Protein in the myeloma patient and it remains a gold standard.

Haematology

Cod: 0714

BLOOD COMPONENT SEPARATION: A NEW EXPERIENCE AT B. P. KOIRALA INSTITUTE OF HEALTH SCIENCES, DHARAN

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BACKGROUND: Blood component separation facility began dispatching its blood component units from 21 April 2013 at the new blood bank at B. P. Koirala Institute of Health Sciences, Dharan. It is presently the only existing blood component separation facility in eastern Nepal. With the supply of blood components being finite and with the added recognition of a high rate of inappropriate use of blood component services around the world, there is a need to monitor and regulate these services.
Objective: Identification of blood component utilisation pattern and demand.

METHODS: The study was retrospective and was conducted at B P Koirala Institute of Health Sciences, Dharan, Nepal for component dispatches from 21 April, 2013 to 1 September, 2013. Requisitions for blood components for patients from various departments were reviewed regarding the department requesting it, the component requested, blood group and socio-demographic profile.

RESULTS: Out of a total of 1240 transfusion units dispatched for different blood components, 657(53.16%) patients were male, 579(46.84%) were female and 4 with no gender identification details. Of the units, 667(53.8%) were PCV (Pack cell volume), 370(29.8%) were PRP (Platelet rich Plasma) and 202(16.3%) were FFP (fresh frozen plasma). 1 unit of cryoprecipitate was dispatched. The largest numbers of requisitions were from the haemodialysis unit (372 PCV). 16 dispatched units were requested from 3 centres outside of BPKIHS. The majority of PCV and FFP requested were of A+ve blood group while the majority of PRP requested were of O+ve blood group. Majority of the requisitions were of 20-29 years age group (24.8%). 94 (7.58%) requisition forms lacked details of requesting department.

CONCLUSIONS: Pack cell volume was the predominant component requested with the haemodialysis unit at the institute making the most requests. Some requisition forms lacked essential details.
Key words: Blood component separation, Fresh frozen plasma, Pack cell volume

Haematology

Cod: 0715

RED CELL SIZE FACTOR IN THE EVALUATION OF IRON DEFICIENT ERYTHROPOIESIS IN HEMODIALYSED PATIENTS

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BACKGROUND: Reticulocyte hemoglobin content (CHr) reported by Siemens analyzers is incorporated to the European Guideline for the monitoring of therapy in dialysed patients. Red blood cell size factor (RSf) provided by Beckman-Coulter systems averages the volume of erythrocytes (MCV) and reticulocytes (MRV). The aims of the study were to investigate the reliability of RSf for assessing iron deficient erythropoiesis in hemodialysed patients, compared to CHr.

METHODS: Fifty five patients (33 male, 22 female, mean age 61.8 years) were included in a longitudinal cohort study with one month of follow up. At the end 220 samples have been processed on LH 750 (Beckman-Coulter) and Advia 2120 (Siemens) analyzers. Correlation coefficients were calculated by Pearson method. Independent samples t test was performed to detect statistical deviations; P< 0.05 were considered statistically significant. Receiver operating characteristic (ROC) curve analysis was applied to verify the diagnostic performance of RSf in the assessment of erythropoietis status. Iron restricted erythropoiesis was defined by CHr < 29 pg. Cohen´s k index for agreement between both parameters were calculated.

RESULTS: Hb (base line mean 114 g/L, SD 11.1 g/L, four weeks 112 g/L SD 20 g/L P=0.34), CHr (base line mean 31.6 pg SD 2.5 pg, four weeks 32.3 pg SD 2.3 pg P=0.23), RSf (base line mean 103.5 fL SD 7.9 fL, four weeks 102.5 fL 5.7 fL P=0.08) remained stable in the follow-up period. Along follow up correlation between CHr and RSf was R=0.774. ROC curve analysis for RSf in the diagnosis of iron restricted erythropoiesis. Area under curve 0.952 (95 % CI 0.902-0.981), sensitivity 80.0 % , specificity 92.5 %, cut off 92.2 fL; using this threshold k=0.71.

CONCLUSIONS: This study shows a good correlation between RSf and CHr. RSf could be a reliable parameter for the study of erythropoiesis in dialysed patients.

Haematology

Cod: 0716

EVALUATION OF UNCERTAINTY FOR HEMATOLOGY LABORATORY BASED ON CLIA, RILIBAK, FRASER AND SIX SIGMA CRITERIAS

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BACKGROUND: Uncertainty is numerical information that complements a result of measurement, indicating the analytical performance of a laboratory. ISO15189 requires that "The laboratory shall determine the uncertainty of results, where relevant and possible". The expression of the uncertainty of a result allows comparison of results from different laboratories, or within a laboratory or with reference values given in specifications or standards. In this study, we aimed to calculate the uncertainty of measurement of our Hematology Laboratory according to EURACHEM/CITAC guide.

METHODS: Study was conducted at the Hematology Laboratory of Department of Medical Biochemistry in Gulhane School of Medicine, Ankara, Turkey. Internal and External Quality Control (IQC and EQC, respectively) results of ABX Pentra DX 120 (Horiba Medical, Montpellier, France) were assessed. 8 parameters (WBC, RBC, Hb, Htc, MCV, MCH, MCHC, platelet count) were evaluated between a period of January 2013-December 2013. With these results, we calculated the analytical performances and uncertainty of measurements of two devices individually and collectively according to EURACHEM/CITAC and AACB guides and compared with reference to CLIA, Rilibak, Fraiser and Six Sigma.

RESULTS: The calculated uncertainty according to AACB of two devices met the expectations in all parameters. However, RBC, Hb and platelet count results of device 1 while only platelet count results of device 2 were determined above Rilibak' criteria. Moreover, calculated total error (TE) rates of Hb, MCV, MCH and MCHC results of both devices were above Fraser' TE rates. Also, only RBC results in device 1 was under 3 sigma, according to six sigma.

CONCLUSIONS: In our study, we determined that one of the devices exhibit better performance than other when calculating individually. Especially in large laboratories, whole blood analysis is carried out more than one device. Calculation of the analytical performance of each instrument individually and collectively, reveal different results in these laboratories.

Haematology

Cod: 0717

RARE CASE OF A PLASMA CELL MYELOMA IN A 12 YEARS OLD PATIENT

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BACKGROUND: The presence of Multiple Myeloma (MM) in young patients aged less than 30 years old is rare. We report the case of a 12 year old boy diagnosed with a plasma cell myeloma moderately differentiated.

METHODS and RESULTS: Case report: twelve years old patient presented asthenia and anorexia of one month duration. He presented hypercalcemia (16.6 mg/dl), increased IgA (4449 mg/dl) and total proteins (12.6 g/dl), normocytic anemia (9.5 g/dl of hemoglobin) and osteolytic bone lesions (punched-out lesions in skull and vertebral compression). Given the suspicion of plasma cell neoplasia the patient was admitted to our hospital to be studied. The new analytic confirmed previous results (IgA=4820 mg/dl, calcium=16.1 mg/dl, hemogloblin=8.0 g/dl) with other significant findings: creatinine 1.61 mg/ dl, albumin 3.2 g/dl, β2microglobulin of 5.98 mg/l, presence of rouleaux formation of erythrocytes in the blood count, ESR of 134 mm/hour, immunoparesis of the other immunoglobulins (IgG=525 mg/dl and IgM=37 mg/dl) and altered ratios of serum free light chains (free kappa=219 mg/dl, free lambda=1.01 mg/dl, ratio=216.83) and Hevylite (IgA-K=66.60 g/l, IgA-L=6.30 g/l, ratio=10.57). In the serum proteinogram it is observed a well-defined monoclonal broad peak in the gamma region (4.34 g/dl correspond to monoclonal component) confirmed by IgA Kappa immunofixation. Bone marrow biopsy detected neoplastic cell proliferation with a CD138+, CD56- and CD20- immunophenotype and Ki67 cell proliferation index up to 50%. Although the patient age does not correspond with MM, the symptomatology and laboratory findings confirmed the diagnosis of MM. Patient was treated with six cycles of chemotherapy based on bortezomib, cyclophosphamide and dexamethasone. At the end of the treatment, the patient achieved a status of complete remission with negative immunofixation, <5% of plasma cells in bone marrow and normal ratios of serum free light chains and Hevylite.

CONCLUSIONS: The diagnosis of MM in patients younger than 30 years is rare. However, this case demonstrates that plasma cell neoplasms should be considered in the differential diagnosis of very young patients presenting severe hypercalcemia and destructive bone lesions to rule out the disease despite the very low incidence in these patients.

Haematology

Cod: 0718

EVALUATION OF PLATELET PARAMETERS IN PATIENTS WITH INCREASED TROPONIN LEVELS BY THE ADVIA® 2120 HEMATOLOGY SYSTEM

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BACKGROUND: Recent improvements in automated blood cell analyzers allow measurement of several platelet parameters, providing additional information for platelet activation. The degree of platelet activation may be assessed by additional platelet indices such as PDW (Platelet distribution width), Large PLT, Mean platelet component (MPC) concentration, MPM (Mean Platelet dry Mass). The aim of the current study was to investigate whether there is an association of platelet count and calculated platelet parameters with a cardiac troponin T (TnT) elevation in patients with acute chest pain.

METHODS: Blood specimens from 70 patients with chest pain and 20 healthy donors was collected in K2EDTA vacutainers and evaluated within one hour by ADVIA 2120 Haematology System. The 2-Dimensional platelet analysis is based on the integrated analysis of erythrocytes and platelet measurements. Patients were classified into three groups: 1) TnT (+)- patients with chest pain and increased troponin T; 2) TnT (-) - patients with chest pain and troponin T in the reference ranges; 3) Controls- healthy subjects free from cardiovascular diseases.

RESULTS: Statistically significant differences in the variances were seen between controls and chest pain study patients for platelet count (F-Test: $p = 0.007$), PCT (F-Test: $p < 0.001$) and large PLT (F-Test: $p = 0.01$). No statistically significant difference in the variance was seen for PMV, PDW, MPM and MPC (F-Test: $p > 0.05$). In contrast, there were statistically significant differences in the mean values observed for MPV (t-test: $p = 0.002$), PDW (t-test: $p = 0.002$) and large PLT (t-test: $p = 0.02$). We found that 82.4% of TnT(+) cases had MPV>8.6fl, 82.4% - PDW>48,6% and 70.6% of them- PMP >2.02 pg. MPV>8.6fl was detected in 35.8% of TnT(-) group and in 40% of controls. PDW>48% was found in 41.5% of TnT(-) group and in 40% of controls. Cut off values for MPM and MPC were 2.02 pg and 24.6 g/dl respectively. There were no differences considered between the study groups.

CONCLUSIONS: We concluded that the most appropriate markers for platelet activation in patients with elevated TnT levels are MPV, PDW and Large PLT count, compared with research calculated parameters MPM and MPC.

Haematology

Cod: 0719

C-REACTIVE PROTEIN IS AN OBJECTIVE MARKER OF INFECTION IN ADULTS WITH ACUTE LYMPHOBLASTIC LEUKEMIA RECEIVING CHEMOTHERAPY

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BACKGROUND: Patients with acute lymphoblastic leukemia (ALL) are immunocompromised due to the neoplastic process, cytostatic chemotherapy (CT), long-term reception of corticosteroids. Timely diagnostics of infectious complications in these patients is difficult, because a severe infection may be asymptomatic or manifest only in the form of fever or malaise. Considering that obtaining of results of microbiological tests may take a long time, there is a need for laboratory markers detecting infectious process at early stage. The aim of the study was to determine of diagnostically significant levels of C-reactive protein (CRP), which can be used for early diagnosis of infectious complications in patients with ALL receiving CT.

METHODS: 34 patients were observed (aged 16–70 years, median 36). All patients at different stages of treatment had infectious complications (86 episodes have been included in the study).

RESULTS: CRP levels in groups of patients with localized infections (such as mucositis, abscess, pneumonia etc.) or fever of undetermined origin (FUO) had no statistical differences ($P>0.05$), but were significant above those in patients without infectious complications ($P<0.05$). CRP concentrations in patients with systemic inflammatory response syndrome (SIRS) and sepsis did not differ ($P>0.05$). At the same time, CRP levels at system infections (SIRS, sepsis) was significant above, than at localized infections ($P<0.001$). CRP levels were: without infection – from 0 to 15 mg/l (median 1 mg/l, n=102 observations), with localized infection or FUO – from 0 to 118 mg/l (median 25 mg/l, n=66), with system infection (SIRS, sepsis) – from 82 to 368 mg/l (median 160 mg/l, n=35). Using receiver operating characteristics (ROC-analysis) we set cut-off points: 11 mg/l for diagnosis of a local infection, 82 mg/l for the systemic infection.Diagnostic sensitivity of these criteria was 92% and 97% respectively, and the specificity - 97% and 97%.

CONCLUSIONS: Thus, CRP is informative biomarker of infection in ALL patients receiving CT. The increase of its level more than 11 mg/l is early evidence of the development of infectious complications. CRP more than 82 mg/l may serve as a tool for the early detection of a systemic infection in these patients.

Haematology

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INVESTIGATION OF MEDICAL INDICATIONS FOR PNH SCREENING EXPERIMENT BY FLOW CYTOMETRY

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BACKGROUND: Paroxysmal Nocturnal Hemoglobinuria (PNH) is an acquired hematopoietic stem cell disorder leading to a partial or absolute deficiency of all glycophosphatidyl-inositol (GPI)-linked proteins. The classical approach to diagnosis of PNH by cytometry involves the loss of at least two GPI-linked antigens on RBCs and neutrophils. Flow cytometry (FCM) is firmly established as the method of choice for screening of PNH. In 2010, technical guidelines were published in order to standardize the diagnosis of PNH by mutiparameter FCM. But there is not a uniform consensus about the medical indications for PNH clone testing by FCM.

METHODS: 3037 individuals were submitted for diagnostic screening of PNH. CD59 was used for RBC analysis based upon physical parameters.We combined FLAER with CD45, CD24, allowing the simultaneous analysis of FLAER and the GPI-linked CD24 on neutrophil and monocyte lineages by CD45 vs side scatter.

RESULTS: PNH clone cells were found in 189/3037(6.2%) cases. Most commonly individuals were screened because of hemoglobinuria (730/3037,24.0%), followed by aplastic anemia (486/3037,16.0%), MDS (448/3037,14.8%), pancytopenia (395/3037,13.0%), hemolytic anemia (298/3037,9.8%), bone marrow failure (211/3037,6.9%), atypical venous thrombosis (64/3037,2.1%), other (405/3037,13.3%). Essentially all patients with classic PNH (95/189,50.3%) report gross hemoglobinuria at some point during the course of their illness. This symptom was absent in patients with PNH-subclincal (eg.PNH/aplastic anemia or PNH/MDS) (9/189,4.8%) because the clone size is often relatively small. PNH clone cell often were found in PNH combined with another specified bone marrow disorder, including AA (58/189) MDS (23/189) pancytopenia (4/189).

CONCLUSIONS: The FCM screening of GPI-deficient cells shows that the rate of positive results is higher when cases were tested because of hemoglobinuria, anemia, thrombosis and pancytopenia, and much higher in cases previously diagnosed of aplastic anemia or MDS, and therefore screening for PNH in patients with aplastic anemia, or MDS even in the absence of clinical evidence of hemolysis, is recommended at diagnosis and at least yearly during follow-up.

Haematology

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ASSOCIATION OF RED BLOOD CELL VOLUME WITH FLUORESCENCE OF CELLS IN EOSIN-5'-MALEIMIDE TEST AND ITS IMPACT ON DIAGNOSIS OF HEREDITARY SPHEROCYTOSIS

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BACKGROUND: EMA (eosin-5'-maleimide) binding test is flow cytometric test used to detect hereditary spherocytosis (HS). The dye is bounded to band 3 protein in erythrocytes membrane proportionately to its amount, what allows to detect its deficiency. To perform the test 6 reference samples of red blood cells and patients red blood cells are needed. Our aim was to investigate how the mean corpuscular volume (MCV) of red blood cells influence on the value of fluorescence of binded EMA dye and how the choice of reference samples impact the test result.

METHODS: EMA test was performed in peripheral blood from 4 patients suspected of hemolytic anemia and 40 reference samples in accordance with procedure described by Ciepiela et al. Reference samples were divided in 3 groups based on MCV of red blood cells. MCV of first group was 81,0 fL (n=18) and mean fluorescence channel (MFC) 34,5, in the second group average MCV was 85,8 fl (n=14) and MFC 36,5, and in third group average MCV was 90,6 fl (n=8) and MFC 38,4. Mean fluorescence channel of EMA-RBC was measured with Cytomics FC500 flow cytometer. Association between MCV and MFC of EMA test was analyzed using Spearman rank correlation coefficient.

RESULTS: The association of MCV and MCF was high. The correlation Spearman coefficient was r=0.59. Only 1 patient of 4 was finally diagnosed as having hereditary spherocytosis (basing on clinical and molecular tests). His MCV was 82,2 fl and MFC 28,1. However, when the MFC of HS patient was compared with reference samples from 1 group his percentage of RBC fluorescence was 82%, which didn't confirm diagnosis of HS. When reference samples with the same MCV as studied patient were used, the percentage of fluorescence was 79% and fully confirmed diagnosis of HS.

CONCLUSIONS: Mean fluorescence of EMA-bind RBC strongly depends on RBC's volume. MCV of reference samples affects actual EMA test results and we strongly recommend that chosen controls should have MCV range of +/- 2 fl compared to MCV of patient RBC's.