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Hematology - Hemostasis

Cod: T193

IDENTIFICATION OF HAEMOGLOBIN VARIANTS DURING GLYCATED HAEMOGLOBIN ANALYSIS

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Background: The presence of a haemoglobin (Hb) variant causes an abnormal chromatogram during glycohaemoglobin A1c (HbA1c) determination by high performance liquid chromatography. The aim of this prospective study is to evaluate the sensitivity and the specificity of the in-house algorithm in the identification of a Hb variant from the pattern and the retention time of the fraction found during the HbA1c analysis on G7 TOSOH analyzer.
Methods: The in-house algorithm for the identification of a Hb variant from the chromatograms, obtained from the HbA1c analysis on G7, is as follows: HbS has a wide pattern (percentage range 32.5% - 36.0%, as heterozygous form) and retention time (RT) interval 1.40 – 1.44 min, with a small peak before the large fraction, HbO-Arab has a wide pattern (percentage range 35.1% - 37.4%, as heterozygous form) and RT interval 1.46 – 1.49 min. Design of the prospective study: one of the author identifies the Hb variant by the visual inspection of chromatograms obtained from HbA1c analysis using the in-house algorithm during one year period. All samples that exhibit an additional peak will be analyzed on G7 β-Thalassaemia Mode TOSOH analyzer and sickle solubility tests will be performed when necessary.
Results: Out of 7991 chromatograms, 154 exhibited an additional peak. 48 peaks are characterized as HbS and 99 as O-Arab by both modes of identification (visual inspection & analysis on G7 β-Thalassaemia mode), (N/score: 147/147; 100% sensitivity & specificity). The remaining 7 chromatograms exhibited an additional peak that did not meet the criteria of the in-house algorithm (1 peak 22.2% and RT 1.39 min, characterized as unknown by both modes, 2 peaks 34.5% & 36.2% and RT 1.26 min, characterized as unknown by the observer and as HbD by the analyzer, 1 peak 12.9% and RT 0.94 min characterized as unknown by both modes (due to aged sample) and 3 peaks <7% and RT 1.46 min (exclusion of HbO-Arab heterozygous due to low percentage; recent blood transfusion history).
Conclusions:The in-house algorithm presented 100% sensitivity and specificity in identification of Hb S and Hb O-Arab (N/score: 147/147). Identification of Hb variants by chromatogram pattern and retention time obtained during HbA1c testing is reliable for those variants that occur very frequently (S, O-Arab) in the population of our geographic area.

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Hematology - Hemostasis

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THE PARAMETERS NEUT-X, NEUT-Y & GRANULARITY INDEX IN REFRACTORY CYTOPENIA WITH MULTILINEAGE DYSPLASIA

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Background: Hypogranularity and dysfunction of neutrophils are the most consistent features of myelodysplasia. Sysmex XE-5000 analyzer provides two parameters for neutrophil activation measurement during routine full blood count using fluorescence flow cytometry; NEUT-X, which represents the inner complexity of neutrophils and is strongly related to granularity and NEUT-Y, which reveals the neutrophil nucleic acid / protein content and is related to production or release of proteins and reactive oxygen intermediates. Granularity Index (GI) is defined based on NEUT-X value. The aim of this study is to evaluate NEUT-X, NEUT-Y & GI in myelodysplastic syndrome subtype "Refractory Cytopenia with Multilineage Dysplasia" (RCMD).
Methods: In order to define the GI, we analyzed 200 samples from healthy individuals on Sysmex XE-5000 analyzer. Based on the resulting NEUT-X mean value (135.9 ch) and the standard deviation (SD = 2.7), we determined the GI by adding or deducting 1 SD from the mean value. The range 133.2 – 138.6 ch corresponds to a normal GI = 0, whereas GI of -1 reflects hypogranulation. The data of the haemograms obtained by full blood count analysis on Sysmex XE-5000 analyzer of 42 patients with RCMD and 66 healthy individuals were collected retrospectively. NEUT-X, NEUT-Y, White Blood Cell count (WBC), # neutrophil count at the time of diagnosis were recorded and the GI was determined. Statistical analysis: Pearson correlation, Mann-Whitney U and Chi-Square tests were applied. Values of $P < 0.05$ were considered to indicate statistical significance.
Results: NEUT-X & NEUT-Y parameters are positively correlated in a statistically significant degree in both RCMD patients and healthy individuals ($r = 0.805$, $P = 0.0001$; $r = 0.545$, $P = 0.0001$, respectively), whereas they are independent of WBC ($P = 0.057$, $P = 0.461$) and # neutrophil count ($P = 0.167$, $P = 0.324$). RCMD patients present statistically significant reduced NEUT-X and NEUT-Y values compared to healthy individuals (mean ± SD: 131.95 ± 4.26 vs 137.17 ± 2.49, $P = 0.0001$ & 35.8 ± 2.8 vs 37.8 ± 2.1, $P = 0.0001$, respectively) and higher proportion of GI < 0 (57.1% vs 4.5%, $P = 0.0001$).
Conclusions: Neutrophil activation as measured by NEUT-X & NEUT-Y is reduced in RCMD myelodysplastic syndrome subtype compared to healthy individuals. The high proportion of GI < 0 seen in RCMD patients indicates the degree of hypogranulation.

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Hematology - Hemostasis

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ABERRANT EXPRESSION OF MYELOID SPECIFIC MARKERS IN ACUTE LYMPHOBLASTIC LEUKEMIA (ALL)

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Background and Purpose:
Documentation of aberrant antigen expression is essential in depicting the neoplastic process and the detection of minimal residual disease. (MRD). Flow cytometry is a significant tool in recognizing aberrant phenotypes. Frequency of aberrant phenotypes varies noticeably in various investigations and its relationship with prognostic issues is quiet controversial. The present study was done to find the frequency of aberrant phenotypes on immunophenotyping in a large series of de novo acute lymphoblastic leukemia (all) and to assess any relationship with initial clinical and hematological features.
Methods:
In the present study, 280 patients of de novo ALL cases were included from two Iranian Immunophenotyping centers during January 2011 to December 2012. The immunophenotype of all cases of ALL was studied using FACSCalibur (BD Biosciences, San Jose, USA).
Results:
Unusual myeloid antigen expression was seen in 38.5% of cases. Most frequent aberrant myeloid antigen was CD13 (31.1%), followed by CD33 (32.2%) and CD117 (24.3%). The expression of CD117 was relatively common in comparison to previous reports which designate its rare expression. Adult T- ALL showed higher expression of CD117 and CD33 than pediatric T-ALL ($p = 0.02$ and 0.04, respectively). Myeloid antigen expression in all cases was associated with lower WBC count ($p<0.05$) and lower number of peripheral blasts ($p<0.05$).
Conclusions:
In summary, CD117 is a fairly commonly expressed myeloid marker contrary to previous reports which denote its rare expression. ALL cases with low blast count and CD34 positivity are more likely to express abnormal myeloid markers.

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Hematology - Hemostasis

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PROGNOSTIC FACTORS IN RELAPSED ACUTE MYELOID LEUKEMIA PATIENTS

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Background and Purpose:
Acute myeloid leukemia (AML) is a heterogeneous and the most frequent type of acute leukemia in adults. Despite recent advances in the characterization of pathogenesis of AML, the cure rate is low. Leukemia relapse the most common cause of treatment failure which occurs due to clonal evolution or clonal escape. The present study is designed to investigate the biological and clinical aspects that influence consequences in patients with AML relapse.
Methods:
A total of 203 AML patients with leukemia were enrolled in the study. They were treated with conventional chemotherapy (CT) and relapse after achieving complete remission (CR) was evaluated by bone marrow (BM) aspirates that were used for conventional Hematoxylin and eosin (H&E) stain, cytogenetics analyses, molecular diagnosis and immunophenotyping. Data from patients who had achieved first CR to assess their prognosis was retrospectively analyzed.
Results:
Overall survival (OS) of the patients was 4.1%±2.6 years. Leukemia progression being the most common cause of death. Patients relapsing before one year and those with adverse cytogenetic and molecular risk factors had significant worse outcomes. A percentage of 45.4% of patients showed phenotypic changes and 30% showed cytogenetic changes at relapse.
Conclusions:
Patients with relapsed acute myeloblastic leukemia have a miserable prognosis, especially those with early relapse and adverse cytogenetic and molecular risk factors. Our results show that the best treatment approach after first and second relapse may differ according to the cytogenetic and molecular risk factors.

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Hematology - Hemostasis

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PLASMINOGEN ACTIVITOR IN YOUNG AFRICAN ADULTS WITH HB-S-GENE TRIAT

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BACKGROUND: The role of plasminogen activator and fibrinolysis in restoring blood flow and vessel integrity within obstructed blood vessels in health and diseases are well elucidated in literatures. The increase incidence and prevalence of cardiovascular diseases amongst Young African Adults motivated this study. However, there are scanty literatures on the possible effect of haemoglobinopahty on blood plasminogen activators and fibrinolysis mechanism in Young African Adults. The strong correlation between cardiovascular diseases and plasminogen activators and blood fibrinolysis is well established in research. The aim of this study is to access plasmonogen activator and fibrinolytic activity in young African Adults with hemogblin genotype AA and AS.
METHODS: A total of 78 apparently healthy Young African Adults volunteers, male 39 and female 39, AA 44 and AS 34 age and sex matched were used fir this study. Their Plasma Fibrinogen Concentration (PFC), Euglobulin Lysis Time (ELT), Plasminogen Activator (PA) and Cellulose Acetate Electrophoresis hemoglobin genotype were analyzed using reference methods.
RESULTS: We observed a significant increase in ELT ($P<0.05$) and significant decrease in PA ($P<0.005$) between HB-genotype AA/AS. There was no age variation in PFC, ELT, and PA between age group 20-24yrs, 25-29yrs, 30-34yrs, and 35-40yrs.
CONCLUSIONS: There is a significant difference in PA and ELT between HB- genotype AA and AS in apparently healthy Young African Adults. We concluded the possible effect of HB- genotype in plasminogen activator and euglobulin lysis time in Young African Adults.

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Hematology - Hemostasis

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THE POSSIBLE EFFECTS OF HB – S- GENE ON PLATELETS COUNT AND PF-3 AVAILABILITY IN AFRICAN WOMEN ON ORAL CONTRACEPTIVES

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BACKGROUND: The role of platelets and platelets factor 3 availability in the maintenance of blood hemostasis and coagulation in health and diseases are well documented in literatures. The usages of various contraceptives by African Women is on the rise. The objectives of this work is aim at investigating the effects of oral contraceptives on platelets count, Platelets factor 3 availability and plasma fibrinogen concentration. The possible effects of HB-S- gene on African women on oral contraceptives and the effect of long-term usages on these parameters

METHODS: A total of 100 apparently healthy females volunteers, 50 controls (AA- 25 and AS-25) and 50 on oral contraceptives (OCP) (AA-25 and AS-25) age matched were used for this study. The platelets count (PC), Plasma Fibrinogen Concentration (PFC), Platelets Factor 3 Availability (PF-3) and Hemoglobin genotype analyzed using Cellulose Acetate Electrophoresis reference methods

RESULTS: We observed a significant decrease in PC ($P<0.05$) and significant increase in PF-3 availability and PFC ($P<0.05$) between controls and OCP. There was a non - significant increase in PC, PFC and PF-3 availability between HB- genotype AA and AS controls, and AA and AS OCP. We also observed a cumulative increase in PFC and PF-3 availability with increase in age and long-term duration of usage of OCP on both AA and AS subjects.

CONCLUSIONS: The use of OCP has a decrease effects on PC and an increase effects on PFC and PF-3 availability, particularly on long-term usage. However, the possible mechanism of HB –S- gene role on PC, PFC and PF-3 availability is not clear in this study.

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Hematology - Hemostasis

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RELATIONSHIP BETWEEN JAK2 V617 MUTATION AND HEMATOLOGIC PARAMETERS IN PHILADELPHIA-NEGATIVE CHRONIC MYELOPROLIFERATIVE NEOPLASMS

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BACKGROUND
V617F mutation of Janus kinase 2 (JAK2) gene is used in the diagnosis of Philadelphia-negative myeloproliferative neoplasms (MPN) such as polycytemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis (PMF). The JAK2-V617F mutation leads to uncontrolled activation of Janus kinase-signal transduction pathway and transcription activators (JAK-STAT) that is a key component regulating cell growth and differentiation. In this study, we aimed to investigate the prevalence of JAK2 V617F mutation and its association with hematologic parameters in PV, ET and PMF patients who have been tested for the mutation.
METHODS
We retrospectively reviewed the records of 168 patients (82 males and 86 females) who were tested for JAK2 V617F mutation from 2013 to 2015 upon request of Hematology Clinic. JAK2 V617F mutation status, white blood cell (WBC) counts, platelet (PLT) counts, hemoglobin (Hb), hematocrit (Hct) levels and demographics of the patients were recorded. JAK2 V617F mutation analysis was performed by real time-polymerase chain reaction (RT-PCR).
RESULTS
JAK2 V617F mutation was detected in 55.9 % of the 168 patients. The JAK2 V617F mutation was observed in 58.2 % of PV cases, in 54.4 % of ET and in 54.5% of PMF cases. All patients were divided into two groups according to mutation being positive and negative. Age, WBC and PLT levels were significantly higher in mutation positive group ($p<0.05$). Age, WBC, Hb, Hct and PLT counts in PV cases with JAK2V617F mutation, age and WBC counts in PMF cases with JAK2V617F mutation were found to be significantly higher compared to mutation negative patients ($p<0.05$).
CONCLUSIONS
JAK2 V617F mutation is a very important parameter in diagnostic and prognostic evaluation. Thus, every patient suspected of having a myeloproliferative neoplasm should be screened for JAK2 V617F mutation. We think possible complications can be prevented by close monitoring and treatment planning in patients with JAK2 V617F mutation.

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Hematology - Hemostasis

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UTILITY OF THROMBOELASTOGRAM (TEG) FOR DESICION MAKING TO PERFORM NEUROAXIAL BLOCK IN THROMBOCYTOPENIC PARTURIENTS

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Background and aims: Gestational thrombocytopenia is believed to be a contraindication to neuroaxial (spinal/epidural) block during labor, fearing occurrence of spinal hematoma with its devastating consequences. No consensus exists on when it is safe to administer neuraxial blockade in women with low platelet count. However, platelets are not the only players in primary hemostasis. Fibrinogen has a pivotal role as well. Hyperfibrinogenemia accompanies the third trimester of pregnancy. We hypothesize that the high plasma concentration of fibrinogen may compensate for the drop in the number of platelets.
Thromboelastogram (TEG) is an point-of-care laboratory test to explore primary hemostasis which is highly dependent on both fibrinogen and platelets. The aim of our study was to use TEG to determine coagulation status in thrombocytopenic parturients.
Materials and methods: During 2010-2015 we performed 107 in parturients in labor with thrombocytopenia of <100,000 mm3. We compared TEG parameters of primary hemostasis [k-kinetic, alpha angle, maximum amplitude (MA)] and fibrinogen plasma levels) between two matched groups, one consisting of parturients with number of platelets between 60,000 to 79,000/mm^3 (group A, n=40) and a second between 80,000 to 99,000/mm^3 (group B, n=67).

Results: The two study groups showed no significant difference in alpha angle and MA, being at upper limit range of normal primary hemostasis. Interestingly, k-kinetic values in group A were higher than in group B (2-tailed Pearson Correlation test), though within normal range which in practice has no meaningful significance.

Discussion and Conclusion: Our findings of upper range normal TEG parameters clearly showed that primary hemostasis remains intact even in moderate to severe thrombocytopenia (platelets count of 60,000/mm^3). This thrombocytopenia is supposedly corrected by the high plasma levels of fibrinogen as found in this study together with increased concentration of other factors (i.e., Von Willebrand). TEG is an accurate and rapid (less than 20 minutes) test to assess primary hemostasis and thus may assist in decision making whether to conduct neuroaxial block in a given thrombocytopenic parturient. However, further studies are warranted to corroborate our results before definite recommendations can be drawn.

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Hematology - Hemostasis

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COMPARISON OF DIAGNOSTIC PERFORMANCE BETWEEN INCREASED BETA2 AND INCREASED COMBINED BETA FRACTION IN SERUM PROTEIN ELECTROPHORESIS: A RETROSPECTIVE AUDIT.

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Background: Serum protein electrophoresis (SPE) separates serum proteins into 5 or 6 fractions and is commonly used as a first line test for detecting and quantifying monoclonal immunoglobulins (MG). Reflex testing of increases in the beta fraction as a combined beta1 and beta2 (↑Beta) or beta2 (↑B2) alone has been a common practice to improve the detection of MG by SPE. However, the relative merits of the two approaches have not been well characterized. The objective of this study was thus to compare the diagnostic performance between these two approaches in the detection of MG.
Methods: We conducted a retrospective study at the Sunnybrook Health Sciences Centre on 3974 consecutive samples with both SPE and immunofixation electrophoresis (IFE) results available within 3 weeks of each other. SPE and IFE were performed on the Sebia Capillarys™ 2 and Hydrasys™ systems respectively. ↑Beta and ↑B2 were defined as the combined beta fraction and the beta2 fraction being greater than 11 and 5 g/L respectively.
Results: Of the 3974 SPE results, 1269 (31.9%) were IFE positive, 150 (3.8%) had ↑Beta while 305 (7.7%) had ↑B2. Within the ↑Beta group, 104 (69.3%) were IFE positive, increasing to 78.6% (81/103) after excluding those with an observable MG or elevated (diffuse) gamma fraction. For the ↑B2, 157 (51.5%) were IFE positive, increasing to 54.3% (114/210) after similar exclusions of those with MG or elevated gamma fraction.
Conclusion: Although ↑Beta or ↑B2 did not have a high prevalence (3.8 and 7.7% respectively) among the patient population studied, their IFE positivity were relatively high (69.3 Vs. 51.5%) and could certainly justify as a basis for further testing. While ↑B2 had a lower IFE positive rate (making it less cost efficient), it did detect more MG (157 Vs 104) in this cohort.

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Hematology - Hemostasis

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ANALYTICAL QUALITY OF PROTHROMBIN TIME AND ACTIVATED PARTIAL THROMBOPLASTIN TIME USING SIX SIGMA

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BACKGROUND: Prothrombin time (PT) and activated partial thromboplastin time (aPTT) are global coagulation tests for diagnosis of coagulation disorders and therapy management. PT expressed as INR is used to monitor warfarin therapy whose desirable value should be 2.5 (range 2-3). It is expected that target value should be reached in 5 days. Although widely used standardization of these tests is main deficiency. The aim of this study was to assess analytical quality of these tests for general use and specifically for monitoring warfarin therapy, and express it as six sigma value.
METHODS: Reagents used for PT and aPTT were: Innovin and Actin FS respectively (Siemens Healthcare Diagnostics, Germany). Six sigma value was calculated using equation: Sigma=(TEa-bias)/CVa. A total allowable error (TEa) criterion was selected from CLIA requirements for quality control (15% for both tests). Bias was assessed from the last external quality assessment (Croatian Center for External Quality Control Assessment: CROQALM): PT=0% and aPTT=3.62%. CVa values were calculated from internal quality control measurements based on 6 month period. Obtained CVa for normal level were: PTsec:4.07%, PT%:7.06%, INR:4.69% and for pathological level: 4.90%, 5.26%, 4.71% respectively. For aPTT expressed in seconds CVa were for normal level 4.15% and for pathological level 3.39%. Additionally six sigma value was calculated using clinical requirement for warfarin therapy 50% change from basal PT value was selected as TEa.
RESULTS: Six sigma for PT normal range was: PTsec: 3.7, PT%:2.1, INR: 3.2, and pathological: 3.1, 2.9 and 3.2 respectively. For aPTT six sigma was 2.7 for normal level and 3.4 for pathological level. When using clinical criterion for PT expressed as INR, six sigma was 10.7.
CONCLUSIONS: When used for achieving desirable INR for warfarin therapy, INR has excellent quality performance. However, our results show that six sigma differ for both PV and aPTT between reference and pathological range, indicating that different internal quality control strategy is required for these tests at different levels of control material.

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Hematology - Hemostasis

Cod: T203

DETERMINATION OF PLASMA TISSUE FACTOR ANTIGEN AND TISSUE FACTOR-BEARING MICROPARTICLES IN HEALTHY SUBJECTS

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Tissue factor (TF) antigen and activity – tissue factor-bearing microparticles (TF-MP) from different origins are thought to be associated with hypercoagulable states. The aim of the present study was to determine the reference range for plasma concentration of TF antigen and TF-MP in a sample of healthy subjects.
Material and methods: To establish the reference range of TF and TF-MP plasma levels and study the impact of sex and age we recruited 110 healthy subjects of Bulgarian nationality aged between 18 and 65. The selection criteria for the reference group were made to comply with the generally approved recommendations of the International Federation of Clinical Chemistry (IFCC). Plasma concentrations of TF and TF-MP were determined using ELISA testing.
Results: The reference ranges given as 95% of the measured values. A reference value was defined for TF antigen plasma levels with 50 (90% CI: 39-56) pmol/l to 194 (90% CI: 185-276) pmol/l, for TF-MP plasma levels with 0.2 (90% CI: 0.1-0.2.) pmol/l to 2.4 (90% CI: 1.9-3.6) pmol/l. We found no sex-related differences in plasma TF and TF-MP concentrations ($P>0.05$), which obviates the need for separate reference intervals for men and women. Single-factor dispersion analysis found no age dependency of levels for TF and TF-MP ($P>0.05$) in the age range 18-65.
Conclusion: The reference values for TF antigen and TF-MP plasma concentrations calculated according to the type of distribution of results can be used as baseline criteria in clinical laboratory studies and for clinical purposes.

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Hematology - Hemostasis

Cod: T204

ANAPLASTIC LARGE-CELL LYMPHOMA WITH ABERRANT EXPRESSION OF CD13:CASE REPORT

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BACKGROUND: Anaplastic large-cell lymphoma (ALCL) is a rare subtype of peripheral T-cell lymphoma. ALCL is usually diagnosed by histologic and immunohistochemical analysis. Multiple studies have shown that flow-cytometric immunophenotyping is useful to aid in diagnosis of ALCL, particularly along with fine-needle aspiration evaluation. Tumor cells in ALCL are by definition CD30+, HLA-DR+ and CD45++, but they can express variable positivity for a number of T cell and non-T cell associated antigens, including CD2, CD3, CD4, CD8, CD25, CD43, CD56, CD13. The myeloid associated antigen CD13 is commonly expressed on neoplastic cells of myelomonocytic origin. CD13 is sensitive but not specific marker of ALCL and should not be misinterpreted as myeloid sarcoma. Considering the disease rarity, the aim of this study was to report a case of ALCL associated with aberrant expression of CD13.
METHODS: A 16-year-old girl presented with a rapidly enlarging supraclavicular mass. A fine needle aspiration of the supraclavicular mass was performed. Specimens were analyzed with cytomorphological and flow cytometric immunophenotyping (FCI) analysis. Expanded FCI antibody panel with 3-color and 4-color direct immunofluorescence staining was performed (CD2, CD3, CD4, CD5, CD7, CD8, CD10, CD11c, CD13, CD14, CD15, CD19, CD20, CD25, CD30, CD33, CD34, CD56, CD64, HLA-DR). Data was analyzed using CellQuest software.
RESULTS: On microscopic examination, cells were atypically large lymphocytes. An aberrant lymphoid population was detected by FCI analysis. Cells were positive for CD45 bright, with most cells falling in the region of monocytes. They were also positve for CD2, CD4, CD7, CD13, CD25, CD30, HLA-DR. ALCL was diagnosed based on FCI findings in conjunction with morphologic evaluation of fine-needle aspirate.
CONCLUSIONS: The case illustrates usefulness of FCI in establishment of the correct diagnosis. FCI is useful to aid in diagnosis of ALCL, particularly along with fine-needle aspiration evaluation.

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Hematology - Hemostasis

Cod: T205

THE ROLE OF VITAMIN B12 AND HOLOTRANSCOBALAMIN IN THE PATHOGENESIS OF ANAEMIA IN PREGNANT WOMEN

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According to different authors, up to 95% of pregnant women have iron deficiency anaemia, but only about a half of them are amenable to correction with iron supplementation. It is possible that vitamin B12 deficiency plays the role in the pathogenesis of anemia in pregnant women. The aim of our study was to investigate the role of vitamin B12 and its active fraction holotranscobalamin in the pathogenesis of anaemia in pregnant women.
The study included 119 pregnant women at the gestational age from 6 to 40 weeks of pregnancy. Levels of holotranscobalamin, total vitamin B12, folic acid and ferritin were determined using "Architect i1000" analyzer (Abbott). Pregnant women were divided into 2 groups – main group ("anaemia") and the comparison group. The main group (n=87) included women with anemia during pregnancy. Its criterion was level of hemoglobin less than 110 g/l. Groups did not differ in age, obstetric and somatic pathology.
In accordance with the selection criterion, in the group of anaemia the level of haemoglobin in the blood (101.3±0.6 g/l) was significantly reduced compared to control (121.7±1.5 g/l). Vitamin B12 and its active fraction holotranscobalamin in the "anaemia" group did not differ significantly compared to control. In the group of women with anemia we revealed a low level of active vitamin B12 in 12% of cases that amounted to 31.7±6.8 pmol/l, and the haemoglobin level in these women was 100.7±5.9 g/L. A positive correlation was found between levels of total vitamin B12 and its active fraction ($p<0.05$), however, the decline in the levels of total vitamin B12 below the reference values did not always coincide with reduced levels of its active fractions. In the "anemia" group a moderate positive correlation between the level of active vitamin B12 and serum iron was also found ($r=0.5$, $p<0.05$).
Conclusion. The lack of a significant reduction of total and active vitamin B12, folate, ferritin and iron in the "anemia" group does not allow us to conclude that the detected hematological changes are the result of a deficiency in the blood of one or more of these substances.

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Hematology - Hemostasis

Cod: T206

HAEMOSTASIS ABNORMALITIES AND INTRAUTERINE GROWTH RETARDATION

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Background: The successful outcome of pregnancy is dependent on the development of adequate placental circulation. Haemostasis abnormalities such as heritable thrombophilias are a group of genetic disorders of bload coagulations that results in increased risk of thrombosis, in pregnancy especialy in intraplacential circulation. That, thrombosis in uteroplacential circulation reduced blood flow throught placenta causing intrauterine growth retardation (IUGR).
The aim of this study was to investigate the occurrence of thrombophilias in IUGR.
Material and methods: Antitrombin, protein C, protein S and resistance to activated protein C (APC-R) were determined in women (n=25) with history of IUGR and controls (20 women with previous normal pregnancies). The age range of patients in the study group was 21-42 years and 19-41 years in control group. Gestation age in group with IUGR was 32,3 ± 3,2 weeks and in control group 37,8 ± 1,4 weeks. Mean values for birth weight in group with IUGR was 2074 ±174 gr and 3421±226 gr in control group. All investigations where made two month after deliveries. Activity of antithrombin protein C were determined by chromogenic methods, but activity of protein S and APC-R were measured by coagulation methods using reagents from Siemens company.
Results: None women in the both groups had antithrombin deficiency. Deficiency of protein C was in 1/25 patients (4% vs none in control group), deficiency of protein S in group with IUGR was 5/25 patients (20% vs none in control group), and APC-R was present in 1/25 women with IUGR (4 % vs none patients in control group).The risk for IUGR in this study was higher in protein S deficiency 7,0, 95% CI 5,40 - 24,95, and simultaneously lower for deficiency of protein C and APC-R were 1,38 , 95% CI 0,65-2,12.
Conclusion: The results indicate that thrombophilias may be one of the risk factor for IUGR.

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Hematology - Hemostasis

Cod: T207

CLINICAL EVALUATION OF THE HYDRAGEL 5 VON WILLEBRAND MULTIMERS KIT OF SEBIA

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Background
The von Willebrand disease (vWD) is the most common inherited bleeding disorder. The underlying cause is a quantitative (type 1 and type 3) or qualitative (type 2) defect of the von Willebrand factor (vWF). The vWF is secreted in the blood flow as a dimeric (Low Molecular Weight: LMW) to a multimeric (High Molecular Weight: HMW) structure. Absence or decreased levels of HMW (as seen in 2A and 2B vWD subtypes) are responsible for the bleeding symptoms.
The treatment of a patient must be adapted depending on its vWD's type/subtype. Different assays are available to type the disease. The electrophoresis of the vWF multimers is one of them and it allows the characterization of the distribution of vWF multimers in the plasma.
Until now, multimers assay was performed using home-made, time consuming and non-standardized methods. In 2016, Sebia has launched a new kit (Hydragel 5 von Willebrand Multimers) on the Hydrasis 2 Scan system. The purpose of this work is to evaluate its performances.

Method
The electrophoresis of vWF has been performed on a Sebia Hydrasis 2 Scan following the recommendations of the manufacturer. Intra-day and inter-day reproducibility have been tested using a normal control and plasma samples from patients presenting different type of vWD. Accuracy of the method has been evaluated using samples from external quality control surveys (ECAT, 3 different surveys). A plasma sample from a type 3 patient has been used to evaluate the inter-sample contamination. Plasma samples from patients presenting the different type of vWD have been analyzed to test the specificity of the assay.

Results
Results showed a complete and partial loss of HMW for the type 2A and type 2B patients, respectively. No multimers were detected in the plasma of type 3 patient.
Our results also showed that the method of Sebia is reproducible and accurate. We did not observe any inter-sample contaminations.

Conclusions
The Hydragel 5 von Willebrand multimers kit of Sebia allows the discrimination of the different subtypes of the vW disease in regards to the distribution of the different multimers of the protein. The method is simple, accurate and fast. It has been ISO15189 accredited in our lab in June 2016.

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Hematology - Hemostasis

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COAGULATION SCREENING IN PREOP PATIENTS

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PT and aPTT are basic hemaostasys tests which are used for screening purposes on Preop Patients. However we sometimes have observed the results as elongated which may be caused by the sensitivity of the reagents, lack of factors or existence of lupus anticoagulant. To overcome this, we perform Mixing test in the lab as a reaction test. Relatively simple procedure used in the hemostasis laboratory as a first-line investigation into the cause of an abnormal screening test, typically a prolonged activated partial thromboplastin time and/or a prolonged prothrombin time. The primary purpose of a mixing test is to guide further investigations. When mixing test results "normalize," this suggests the test plasma is deficient in clotting factors and thus specific factor assays can be performed to determine which are reduced.When the mixing test result does not "normalize," this suggests the presence of an inhibitor or other type of interference (e.g. the presence of an anticoagulant such as high-dose heparin), and so the laboratory needs to determine if this is a reagents, lupus anticoagulant or a specific coagulation factor inhibitor, or another type of inhibitör, the appropriate performance and interpretation of mixing tests is advantageous for the laboratory. Moreover, the correct laboratory approach is also clinically relevant, as patient management is ultimately affected, and an incorrect interpretation may lead to inappropriate therapies being established. As Ankara Numune Education and Research Hospital Hematology Lab, when we look for the Mixing Tests and their resultswe observe that we have 30,000 Patient annually who have PT or aPTT. Our Lab performed 244 (0,81%) mixing tests for aPTT and 321 (1,7%) for PT. All the mixing tests performed in our laboratory were repeated twice in different times by using different system and reactive. 90% of elongated PT cases were found normal whereas 50% of aPTT case were normal. İt is recomended to perform such tests with different methods before the reaction tests in the Preop Patients who have elongated PT and aPTT. İn case it is again found elongated by the different test we propose to investigate in the loght of mixing test result and patient’s clinical history, the lack of factor, existence of inhobotor and existance of anticoalgulant

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Hematology - Hemostasis

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LACTATE DEHYDROGENASE LIKE DIAGNOSTIC MARKER IN HIGH-GRADE B-CELL LYMPHOMA.

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INTRODUCTION

High-grade B-cell lymphoma (HGBCL), like Burkitt's lymphoma or diffuse large B-cell lymphoma, is a type of non-Hodgkin lymphoma that is developed from B lymphocytes.
The high proliferative rate of these lymphomas combined with a high rate of "spontaneous" cell death in the tumor explain the importance of providing a diagnostic biomarker to differentiate HGBCL from other acute hematologic diseases such as acute leukemias (AL).
The level of lactate dehydrogenase (LDH) in plasma has been eventually used to evaluate the prognosis in patients with HGBCL but its use in the diagnosis hasn't been studied yet.

OBJECTIVE
Investigating if the level of plasma LDH is different between the groups of patients with HGBCL and AL.
Finding a cut off that discriminates both groups of patients with the highest diagnostic sensitivity and specificity.

METHODS

This is a retrospective study that was conducted from January 2012 to October 2016.
We looked for all the plasma samples with LDH > 5000 U/L from hematologic patients discarding patients without neoplasic illness (hemolytic anemia, megaloblastic anemia, etc).

31 patients were admitted in the study: 18 with HGBCL, and the other 13 patients with AL.

The Kruskal Wallis rank test was used to analyse statistically significant difference between HGBCL and AL. To determinate the cut-off of this biomarker the receiver operating characteristic curve (ROC) was used.

RESULTS

The average of plasma LDH levels in HGBCL group was higher (12744.500 U/L) than AL (7564,083 U/L).
There were statistically significant differences between both groups (p=0,008141) and the cut-off for plasma LDH was set at 7843 U/L providing a diagnostic sensitivity and specificity of 94,6 % and 60,96% respectively.

CONCLUSIONS

In summary, our data suggest an association among the high plasma LDH levels and High-grade B-cell lymphoma (Burkitt's lymphoma or diffuse large B-cell lymphoma).

With only plasma LDH level is possible to discriminate between HGBCL and AL with a cut off 7843 U/L with a very good sensitivity (94.6%). However, the specificity must be improved (60.96%) combining with other biochemical and hematological biomarkers.

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Hematology - Hemostasis

Cod: T210

CCL-2 IS A KIT D816V-DEPENDENT MODULATOR OF THE BONE MARROW MICROENVIRONMENT IN SYSTEMIC MASTOCYTOSIS

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Systemic mastocytosis (SM) is characterized by abnormal accumulation of neoplastic mast cells harboring the activating KIT mutation D816V in the bone marrow and other internal organs. Similar to other myeloproliferative neoplasms, increased production of pro-fibrogenic and angiogenic cytokines and related alterations of the bone marrow microenvironment are commonly found in SM. However, only little is known about mechanisms and effector molecules triggering fibrosis and angiogenesis in SM. The aim of our study was to identify cytokines that are produced in neoplastic MC in a KIT-dependent manner and contribute to abnormal angiogenesis and fibrosis in SM. Thus, we screened for KIT D816V-dependent production of cytokines relevant to inflammation and microenvironment alterations in MPN. Various cytokines including CCL-2, a CC chemokine that recruits inflammatory cells to sites of inflammation and enhances angiogenesis, were highly upregulated in growth factor-dependent human cells after lentiviral transduction with KIT D816V. Correspondingly, the KIT-targeting drug midostaurin ($p=<0.001$) and RNAi-mediated knockdown of KIT ($p=<0.0001$) reduced expression of CCL-2. We also found that NFκB contributes to KIT-dependent upregulation of CCL-2 in mast cells. In addition, CCL-2 secreted by KIT D816V+ mast cells was found to promote the migration of human endothelial cells in boyden chamber assay (126% of control, $p<0.05$) as well as in wound healing assay (214% of control, $p<0.01$). Furthermore, knockdown of CCL-2 in neoplastic mast cells resulted in reduced microvessel density ($p=<0.001$) and reduced tumor growth ($p=<0.05$) in NSG mice in vivo, compared to CCL-2-expressing cells. Finally, we measured CCL-2 serum concentrations in patients with SM and found that CCL-2 levels were significantly elevated in mastocytosis patients compared to controls ($p=0.0048$). CCL-2 serum levels were higher in patients with advanced SM and were found to correlate with poor survival ($p=0.0001$). In summary, we have identified CCL-2 as a novel, KIT D816V-dependent, key regulator of vascular cell migration and angiogenesis in SM. CCL-2 expression correlates with disease severity and prognosis.

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Hematology - Hemostasis

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HEMATOLOGICAL SAMPLE TESTING IN NEW MINICOLLECT® BLOOD COLLECTION TUBES

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Background: Where small sample volumes are critical, especially for infants, elderly or obese patients, the new MiniCollect tube allows the highest flexibility and accuracy by collecting blood in unprecedented simplicity. MiniCollect® K2EDTA and K3EDTA Blood Collection Tubes are used to collect, transport, store and evaluate capillary blood specimens for hematology tests.
Methods: A study was done at Steyr Hospital (Austria) using MiniCollect tubes with the old design vs. new design. Altogether, 65 hospitalized and 50 healthy subjects were recruited. Informed consent was given by all donors and the study was approved by EC Upper Austria. Directly after blood collection, the tubes were inverted 8 times and processed according to the IFU for MiniCollect tubes. Complete blood counts including 15 parameters were tested using a DxH800 (Beckman Coulter). Analysis was done with the instrument's accompanying reagents.
Results: Evaluation of all clinical data and deviations was done on the basis of the maximum allowed deviation for a single value according to the guidelines of the German Association of Quality Assurance of Laboratory Testing (Rilibäk). The utilization of tubes with old and new design did not reveal any clinically nor statistically significant deviations ($p<0.05$). Comparing the initial values of the old and new design, both EDTA tubes resulted in a highest deviation of 3.0%. Comparable highest deviations for initial values in relation to 48h values were obtained for K2EDTA (5.3%) and K3EDTA (6.4%).
Conclusion: From a clinical perspective, the MiniCollect K2EDTA and K3EDTA tubes with the new design are substantially equivalent to the tubes with the old design. The newly designed tubes provide an essentially enhanced blood collection device for skin-puncture testing. As the fundamental advantage is the guarantee of the sample integrity for high quality results in case of critical sample collections and transport of the tubes, the supporting information and data obtained from adult populations are more than adequate to establish safety and effectiveness for the patient indication.

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Hematology - Hemostasis

Cod: T212

AN AUTOMATED APPROACH TO PLEURAL EFFUSION CELL COUNT IN THE ONCOLOGY SETTING

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The body fluid mode of the Sysmex XN-2000 haematology analyser differentiates cells into mononuclear and polymorphonuclear white blood cells (WBC) and high-fluorescent cells (HFC). The aim of this study is to evaluate the instrument's performance and performance of the HFC count for detecting malignant cells in pleural effusions.
Fifty eight pleural effusions (17 malignant) were analysed on the Sysmex XN body fluid mode. All samples employed in this study derived from those sent to the laboratory for routine clinical analysis and were collected in K_2-EDTA tubes. WBC and RBC (red blood cell) counts and WBC differential counts determined using an automated method were performed on the remaining samples.
To determine the instrument's WBC within-run precision and precision profile serially diluted pleural effusions were used. The samples were 10 times consecutively assessed on the analyser. The mean WBC count of each dilution was plotted against the coefficient of variation (CV). The point where the CV exceeded 20% was arbitrarily defined as the lower limit of quantitation (LLoQ). Between-run precision was assessed by analysing two levels of control samples over 23 days. For evaluating linearity, pleural fluids with high WBC or RBC counts were serially diluted with system diluent to relevant concentrations. All samples were measured five times and the means were compared with the expected cell counts. HFC were measured as a relative count (HFC/100 WBC) and absolute count (HFC/μL). All samples were microscopically screened on cytospin slides for the presence of malignant cells which were confirmed by immunocytochemistry or flow cytometry analyses.
Precision profile for WBC count revealed LLoQ of 0.007 x 10^9/L. The between-run precision was <4.9% for WBC, <2.9% for RBC, <10.1% for MN and <8.1% PMN for both control levels. The linearity was proven to be excellent for pleural effusions (r= 1.00). The median number of HFC was significantly higher (P<0.0001) in those with malignant cells (17.3/100 WBC, range: 1.5-140.6) than in those without (6.0/100 WBC, range 0.0-54.1). Area under the ROC curve for relative HFC count was 0.727 (95% CI= 0.590-0.865).
In conclusion, the data presented in this study demonstrates that the Sysmex XN-2000 body fluid mode is reliable and accurate for counting WBC (differential) and RBC in pleural effusions. A cutoff level of 9% HFC showed the best performance to predict malignancy, with 88.2% sensitivity and 63.4% specifity.

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Hematology - Hemostasis

Cod: T213

EFFECT OF CIGARETTE SMOKING ON HEMATOLOGICAL PARAMETERS IN HEALTHY POPULATION

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Objective: Tobacco cigarette smoking is one of the major leading causes of death throughout the world. Smoking has both acute and chronic effect on hematological parameters. The aim of the present study was to assess the extent of adverse effects of cigarette smoking on biochemical characteristics in healthy smokers.
Subjects and Method: One hundred and fifty six subjects participated in this study, 56 smokers and 100 nonsmokers. The smokers were regularly consuming 10-20 cigarettes per day for at least 3 years. Complete blood cell count was analyzed by CELL-DYN 3700 fully automatic hematological analyzer.
Results: The smokers had significantly higher levels of white blood cell (WBC) ($p<0,001$), hemoglobin ($p=0,042$), MCV ($p=0,001$) and MCHC ($p<0,001$). All other measured parameters did not differ significantly. Cigarette smoking caused a significant increase ($p<0,001$) in RBC, WBC ($p=0,040$) hemoglobin ($p<0,001$), hematocrit ($p=0,047$) and MCH ($p<0,001$) in males in comparison to female smokers.
Conclusion: In conclusion, our study showed that continuous cigarette smoking has severe adverse affects on hematological parameters (e.g., hemoglobin, WBC count, MCV and MCHC, RBC, hematocrit) and these alterations might be associated with a greater risk for developing atherosclerosis, polycythemia vera, chronic obstructive pulmonary disease and/or cardiovascular diseases.

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Hematology - Hemostasis

Cod: T214

PROCALCITONIN AND C-REACTIVE PROTEIN AS EARLY MARKERS OF BACTERIAL INFECTIONS AMONG PATIENT WITH HEMATOLOGICAL MALIGNANCIES RECEIVING CHEMOTHERAPY

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Background: The immune system of patients with hematological malignancies is suppressed during chemotherapy. This renders them vulnerable to frequent infections especially bacterial. Timely diagnosis of these infections is difficult, because a severe infection may be asymptomatic or manifest only in the form of fever or malaise. There is need to come up with lab markers that can detect an infectious process at an early stage. The aim of this study was to determine the value of using Procalcitonin (PCT) and C reactive protein (CRP), for early diagnosis of infection in patients with hematological malignancies receiving chemotherapy.
Method: This was a cross sectional study consisting of sixty eight (68) patients with hematological malignancies. Data from each participant including sex, age, clinical and laboratory presentation were collected after obtaining informed consent. Then Specimens collected for measurement of PCT, CRP and bacteriological analysis. Patients were divided into two groups; those with a culture positive and negative result. PCT and CRP concentrations were compared between groups using t-test and non parametric statistical tests respectively including ROC curve, sensitivity, specificity, likelihood ratio, and Spearman's correlation coefficient.
Results: A total of 14 (20.6%) microorganisms were isolated, of which 10 were gram-positive bacteria and 4 were gram-negative bacilli. The mean values of PCT which were 6.1ng/mL in the bacteremia group and 5.1ng/mL in the non-bacteremia group, p=0.023 and median CRP values were 24.2 (6.43-48.15) in the bacteremia and 23.5 (6.03-75.44) in the non-bacteremia group, p=0.832. The area under curves was 0.52 (95% CI=0.57-0.84) for CRP and 0.70 (95% CI=0.35-0.69) for PCT. PCT value of greater than 5.1ng/mL is diagnostic for infections (sensitivity 71%, specificity 65%) while that of CRP was 21mg/mL with the sensitivity and specificity of 64% and 44% respectively. high levels of PCT as well as fever were significantly associated with bacteremia.
Conclusion: PCT measurement can contribute to the early diagnosis of bacterial infection in patients with hematological malignancy. In contrast the sensitivity and specificity for CRP are too low to safely rely on it as a marker of infection in patients with hematological malignancy.

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Hematology - Hemostasis

Cod: T215

CHANGES IN BLOOD VISCOSITY, YIELD STRESS, AND ECHOCARDIOGRAPHIC PARAMETERS BEFORE AND AFTER HEMODIALYSIS

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Background: Increased blood viscosity has been reported as a cardiovascular risk factor, and hemodialysis (HD) has been known to accelerate cardiovascular and cerebrovascular pathogenesis in patients with end-stage renal disease (ESRD). We investigated the changes in diastolic blood viscosity (DBV), yield stress (YS), and echocardiographic parameters before and after HD.
Methods: DBV was measured in 61 ESRD patients undergoing regular HD, using the Hemovister (Ubiosis, Gyeonggi-do, Korea), and YS was calculated before and after HD. Echocardiography was also performed before and after HD. Correlations between delta YS (%), delta DBV (%), delta laboratory parameters(%), Kt/V(as well known as a dialysis adequacy parameter) and delta echocardiographic parameters (%) were analyzed, and their differences were compared in the patient groups based on delta YS (%).
Results: Delta YS (%) showed very high correlation with delta DBV (%) ($r = 0.9685$) and showed low negative correlations with delta E velocity(%) and delta A velocity(%)($r=-0.3217$;$r=-0.4111$). Patients were divided into three groups based on delta YS (%) with cut-offs of 0% and 100%. Delta DBV (%), delta E velocity(%), and delta A velocity (%) differed significantly in the three patient groups of delta YS (%) (-3.60 vs. 25.52 vs. 83.93, $P < 0.0001$;-12.27 vs. -25.83 vs. -34.21, $P=0.009$; 0.16 vs. -15.64 vs. -24.51, $P = 0.001$). Delta BUN(%), delta beta2 microglobulin(%), delta NT-pro BNP(%), and Kt/V did not showed significant difference in the three patient groups of delta YS(%).
Conclusions: The changes in DBV, YS, and echocardiographic parameters are variable in HD patients. These findings may provide fundamental tools to classify HD patients with different cardiovascular risks.

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Hematology - Hemostasis

Cod: T216

HETEROZYGOUS HEMOGLOBIN E – A CASE PRESENTATION

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Introduction: Hemoglobin E (HbE) is estimated to affect at least one million people worldwide. Carrier frequency is highest in Southeast Asia, reaching as high as 60% in parts of Thailand, Laos, and Cambodia. The aim of this study is to present a case of HbE trait, detected in a Greek navy recruit.

Materials and methods: Patient haemogram was evaluated on the Sysmex k1000 fully automated analyzer. Serum iron and ferritin levels were measured on the Olympus AU640 biochemistry analyzer. Alkaline and acid hemoglobin electrophoresis was performed with the SEBIA Hydrasys fully automated electrophoresis system.

Results: Hematocrit 43,1%; hemoglobin 15,4 g/dL; red blood count 5,44; MCV 79,2; MCH 26,3; MCHC 35,7; ferritin 18 ng/mL; iron 40 mg/dL. A few target cells were present in the blood smear Electrophoretic pattern showed HbE=41,9%, HbF=0,8%, HbA_2=2,2%, HbA=55,1%. Osmotic fragility curves were moderately shifted to the right, indicating slightly decreased osmotic fragility.

Discussion: HbE is characterised by the substitution of lysine for glutamic acid at position 26 of the β-globin chain. HbE heterozygotes are clinically normal, with minimal changes in blood counts and erythrocyte indices. Red cell morphology is similar to that in thalassaemia minor with normocytic or slightly microcytic red cells. Although HbE trait does not cause significant clinical problems, its interactions with various forms of α and β thalassemia produce a very wide range of clinical syndromes of varying severity.

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Hematology - Hemostasis

Cod: T217

SIGNIFICANCE OF MONOCLONAL ANTIBODY PANEL FOR MATURE B CELL NEOPLASMS IN FLOW CYTOMETRIC EXAMINATION OF CHRONIC LYMPHOPROLIFERATIVE DISEASES

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Background: Mature B cell neoplasms comprise over 90% of lymphoid neoplasms worldwide. Together with morphology, flow cytometry immunophenotyping is essential for diagnosis of these diseases. The aim of this study is to examine concordance of working diagnosis with immunophenotyping results in patients with suspicion on mature B cell neoplasm.
Methods: Examination included 125 patients, divided in 3 groups on diagnosis founded by hematologist: CLL , NHL and other (descriptive diagnosis).Sample was K2EDTA peripheral blood, diluted with phosphate buffer so that contains million leukocytes/100 µl and added in tubes with monoclonal antibodies. After incubation and lysing, samples were washed and fixed. 4-color antibody panel for B chronic lymphoproliferation was applied. Analysis performed on FACS Canto II flow cytometer, DIVA software. Samples scored by 5 points score (CD5, CD23, FMC7,CD79b and surface immunoglobulin chains)
Results:83 samples came with diagnosis CLL. In 64 CLL was confirmed. 14 immunophenotipization results that did not match CLL. 5 were CLL vs MCL. 13 samples came to laboratory with diagnosis NHL, 5 confirmed. 29 samples came with descriptive diagnosis; lymphocytosis, lymphadenopathy; splenomegaly, pancytopenia, chronic vs acute leucosis. 4 were excluded because of inadequate indication. Summary for the first 2 groups, 72.9% immunophenotypisation were in concordance with working diagnosis, 7.3% partially, 19% showed different diagnosis. In 84.3 patients suspected to have mature B cell neoplasm by using panel for chronic lymphoproliferation diagnosis was established, 15,7% needed further investigation in specific direction.
Conclusins: By using flow cytometry, missclasification and inadequate therapy was prevented for significant number of patients and diagnosis established for those with descriptive diagnosis. Panel for chronic lymphoproliferative diseases is also useful for differential diagnostic exclusion of chronic lymphoproliferative disease and pointing towards specified direction, e.g. acute leucosis, T, B, NK lymphoproliferative disorders or to confirm normal B cell phenotype.

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Hematology - Hemostasis

Cod: T218

DIAGNOSIS OF HEMOGLOBIN DISORDERS BY HPLC

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BACKGROUND: Inherited abnormalities of hemoglobin synthesis include many disorders ranging from thalassemia syndromes to structurally abnormal hemoglobin variants. The aim of this study was to determine the prevalence of thalassemias and hemoglobinopathies in Greek patients.

MATERIAL AND METHODS: A prospective study was undertaken in which 2650 cases were included over a period of 3 years. The venous blood samples were analyzed for complete blood count, serum iron and ferritin levels. High-performance liquid chromatography (HPLC) was performed on the samples with Menarini Adams HA-8160 automated analyzer. Confirmatory tests and electrophoresis were done whenever required.

RESULTS: Abnormal hemoglobin fractions on HPLC were seen in 326 of the 2650 cases (12.3%) Of these, the beta thalassemia trait was the predominant abnormality with a total of 270 cases (10.2%). A cut off value of >3.0% was considered for diagnosis of beta thalassemia trait. The rest comprised of HbS trait (19 cases; 0.7%), HbS trait /beta thal trait (18 cases; 0.68%), hereditary Hb F persistence (15 cases; 0.57%), Hb Lepore trait (1 case; 0.038%), HbD trait (2 cases; 0.075%) and HbD trait/HbG trait (1 case;0.038%).

CONCLUSION: Hemoglobinopathies are one of the major public health problems in the mediterranean countries. Premarital screening is an important measure in order to prevent birth of children with severe hemoglobin disorders. HPLC forms a rapid, reliable and accurate tool for the detection of various hemoglobin disorders. Moreover, it is combined with complete automation, simplicity of the sample preparation, superior resolution, relatively high sensitivity or specificity and accurate quantitation of hemoglobin concentration.

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Hematology - Hemostasis

Cod: T219

DO THE ROUTINE HAEMATOLOGY RESULTS CAN BE AFFECTED BY THE LONGEST FRENCH PNEUMATIC TUBE SYSTEM ?

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BACKGROUND:
The use of Pneumatic Tube System (PTS) is increasing more and more in the hospitals labs. It reduces the delivery time as well as the cost. As part of the reorganization of the biology network in the second largest university hospital in France (Hospices Civils de Lyon), one haematology labs has been moved from Hopital Edouard Herriot to a central lab located in the East Hospital Complex approximately 2000 meters away.
A direct underground connection through a nine pipes PTS measuring 2130 meters long has been implemented to dispatch patient samples to the central labs. This PTS is the longest one in France.
Prior to the start-up of the installation, several tests were carried-out and the PTS was tested at two different speeds: 4m/s and 5m/s (i.e. 16 km/h and 18 km/h).Time required to reach the arrival station at these speeds are 7 and 8 minutes respectively and a sample can be sent every 75 seconds. Maximum acceleration is about 2g and then deceleration is progressive all the length long.

METHODS:
Three blood samples of ten healthy volunteers were collected: one was dispatch by road (RT) and two samples were transported through the PTS at the two different speeds (S1=4m/s; S2=5m/s). All samples were analyzed at the same time on CELL-DYN® Sapphire (Abbott) to evaluate the potential consequences of PTS on routine hematology results, including white cell differential parameters, platelet indices and red cell fragmentation.

RESULTS:
None of these results evidenced any statistical difference between samples by road versus PTS speed 1 (n=10; RT vs S1) and samples dispatched by road versus PTS speed 2 (n=10; RT vs S2) (p-value>0.05 ; Wilcoxon test).

CONCLUSIONS:
PTS can be used safely to transport Complete Blood Cell Count samples since it showed no impact on the results of routine haematology parameters. We concluded that the PTS was qualified to transport samples to the central Lab, over a length of 2130 meters.

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Hematology - Hemostasis

Cod: T220

ASSOCIATION BETWEEN RETICULATED PLATELETS AND METABOLIC PROFILE INDICATORS IN TYPE 2 DIABETES MELLITUS

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Background: Type 2 diabetes mellitus is associated with increased platelet turnover and increased number of immature, reticulated platelets (RP). Aim of this study was to evaluate association between percentage of RP, marker of platelet turnover, and the metabolic profile indicators in patients with type 2 diabetes mellitus (T2DM).
Methods: The study included 43 patients, 33 to 70 years of age, previously diagnosed with T2DM. Control group included 30 age and sex matched healthy participants. All subjects underwent anthropometric measurements and laboratory analysis of blood samples on automated analyzers with determining the parameters of glucose metabolism, inflammation parameters and platelet parameters. Reticulated platelets (% of total platelets), %RP, was measured by an automated hematology analyzer CELL-DYN Sapphire, Abbott Lab., using flow cytometry assay. Homeostasis model assessment-estimated insulin resistance index (HOMA-IR), Body mass index (BMI) and Waist to Height Ratio (WHtR) were calculated.
Results: There are statistically significant higher values of BMI (30.8vs.24.8;$p<0.001$), Waist circumference (108.8vs.87.1;$p<0.001$), WHtR (0.63vs.0.50;$p<0.001$), HOMA-IR (6.14vs.2.41;$p<0.001$), HbA1c (51.0vs.34.7;$p<0.001$), fibrinogen (3.77vs.3.10;$p<0.01$) and hsCRP (4.14vs.0.77;$p<0.001$) in diabetics compared to healthy controls. %RP was significantly higher in diabetic patients (3.47vs.2.29;$p<0.001$) than in controls. %RP strongly positively correlated with BMI ($r=0.365$;$p=0.016$), WC ($r=0.435$;$p=0.004$), WHtR ($r=0.373$;$p=0.014$), HOMA-IR ($r=0.409$;$p=0.006$), HbA1c ($r=0.314$;$p=0.040$), fibrinogen ($r=0.312$;$p=0.042$) and hsCRP ($r=0.374$;$p=0.014$), in diabetic patients.
Conclusions: Correlation of %RP with metabolic profile indicators is a reflection of overall influence of poor metabolic profile on high platelet turnover in type 2 diabetes mellitus.

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Hematology - Hemostasis

Cod: T221

COMPARISON OF TWO METHODS FOR DETERMINATION OF ERYTHROCYTE SEDIMENTATION RATE

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Background: Westergren method is a benchmark for determination of erythrocyte sedimentation rate (ESR), but the TEST1 is an automated method for exact measurement of ESR. Aim of the study was to compare Westergren method versus the TEST1 technique for determining the ESR.
Methods: Standard Westergren method is a procedure for estimating the ESR by mixing venous blood with an aqueous solution of sodium citrate and allowing it to stand in an upright position for an hour in order to read ESR. The TEST1 (Alifax Roller 20, Padova, Italy) is an automated method that measures the kinetics of blood sedimentation phenomenon. This method studies the sedimentation and aggregation capacity of the red blood cells via optical density by reading every sample 1000 times in 20 seconds. Intermethod comparison was determined by using 122 randomly choosed, blood samples from patients with various inflammatory diseses and healthy subjects, using Passing-Bablok analysis. The correlations of TEST 1 data and those of the Westergren method were evaluated by linear regression and paired t tests. Blood samples for Westergren method were collected in Westergren sodium citrate 4:1 ESR vacutainer tubes and for TEST1 method, blood was collected in K3EDTA vacutainer tubes. All blood samples were processed within 4 hours from blood collection. Statistical analysis of obtained results was performed by MedCalc statistical software.
Results: The mean ± SD ESR was 30.93 ± 16.58 mm/h (range, 9-84 mm/h) for TEST 1 and was 33.41± 23.85 mm/h (range, 10–100 mm/h) for the Westergren ($P < 0.05$). There was a significant correlation between TEST1 and Westergren measurements ($r 2 = 0.7076$;$P < 0.001$). Comparison between Westergren method and TEST1 by Passing Bablok analysis (slope=0.72, 95%CI 0.63 to 0.82; intercept=7.26 95%CI 4.83 to 9.04) showed that there are proportional and constant differences between the results obtained by these two methods.
Conclusions: A method comparison between Westergren method and TEST1 showed poor agreement indicating that these two methods can’t be used interchangeably for determining the ESR.

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Hematology - Hemostasis

Cod: T222

SEBIA HYDRAGEL 5 VON WILLEBRAND MULTIMERS - A NEW AND RAPID VON WILLEBRAND FACTOR (VWF) MULTIMER SCREENING METHOD TO AID SUBTYPING OF TYPE 2 VON WILLEBRAND DISEASE (VWD)

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Background
Analysis of VWF multimers is essential for diagnosis and classification of VWD. Reduction of high molecular weight multimers (HMWM) is observed in types 2A and 2B VWD whilst a normal pattern is seen in types 2M and 2N. The suitability of the Hydragel 5 von Willebrand multimers (Sebia, France) for subtyping of type 2 VWD was evaluated as part of a larger ongoing comparison study. Method The multimer pattern of 54 samples from previously diagnosed VWD patients (Types 2A n= 25, 2B n= 6, 2M n= 18, type 2N n= 5) were analysed by two methods. The semi-automatic Hydragel 5 von Willebrand Multimers, using Hydrasys 2 scan instrumentation involved electrophoresis on agarose gel, immunofixation with anti-VWF, visualisation using peroxidase-labelled antibody and densitometry of the multimers. The in-house method involved agarose gel electrophoresis and visualisation using alkaline phosphatase-conjugated antibody.
Results
All type 2A samples showed a reduction in HMWM with Hydragel 5 von Willebrand multimers. One individual produced a normal multimer pattern using the in-house method, whereas other family members showed a reduction in both.17 type 2M samples had normal multimers, however, a number of these showed flattened HMWM peaks with densitometry. One 2M sample with a very low VWF level showed normal pattern with the in-house method but reduction of HMWM with the Hydragel 5 von Willebrand Multimers. The analysis of multimers using the Hydragel method is not recommended in individuals with very low VWF levels. All type 2B and type 2N samples produced expected multimer patterns with both methods.
Conclusions
52/54 gave the expected multimer pattern with the Hydragel 5 von Willebrand multimers. Densitometry, available with the Hydragel 5 von Willebrand multimers, highlighted heterogeneity in HMWM concentration between family members with type 2A. The densitometry of some of the 2M samples appeared to have a slight reduction of HMWM with flattened peaks. This may prove to be a unique pattern for some type 2M individuals using the Hydragel 5 von Willebrand multimers and may be related to the genetic mutation present. The Hydragel 5 von Willebrand multimers is suitable for use in the classification of type 2 VWD with the helpful addition of densitometry.

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Hematology - Hemostasis

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IDENTIFICATION AND QUANTIFICATION OF URINARY MONOCLONAL PROTEINS BY CAPILLARY ELECTROPHORESIS IN AL AMYLOIDOSIS

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Identification and quantification of urinary monoclonal proteins (uMPs) is fundamental in the diagnosis and monitoring of monoclonal gammopathies (Kyle et al. Leukemia 2010). We prospectively assessed the performance of the Sebia Capillarys urine protein capillary electrophoresis (UPCE) and immunotyping in 75 patients with AL amyloidosis. Samples were tested with: homemade high-resolution agarose gel immunofixation electrophoresis (hr-IFE) of serum and concentrated (10 times) urine; commercial semi-automated agarose gel immunofixation of urine (Sebia Hydragel BJ on Hydrasys 2, SHBJ); UPCE and immunotyping (Sebia Capillarys 2 Flex Piercing Urine); quantification of circulating free light chains (FLC) by Freelite and N latex FLC. Urinary MPs were quantified using Sebia Phoresis software tools. Sixty-eight patients in whom uMPs were detected by hr-IFE were included in the study. A uMP was detected by UPCE in 62 cases (91%), and was quantifiable in 55 (81%). The median uMP excretion was 130 mg/24h (range 10-1610). Nine of the 12 patients with dFLC<50 mg/L (Freelite) had a quantifiable uMP (median 90 mg/24h). The uMP was also quantifiable on SHBJ in 51 patients (75%). There was a good correlation between measurements of uMP excretion on UPCE and SHBJ (Pearson's r = 0.87, 95%CI 0.78-0.92). So far, 16 patients with quantifiable uMP and dFLC (Freelite) >50 mg/L were treated and had response data at 3 months. Five subjects responded with a median 69% dFLC decrease (range 51-90%). In all of them uMP excretion decreased (median 100%, range 30-100%). Among non-responders, only one had a relevant reduction in uMP (from 740 to 250 mg/24h, dFLC from 746 to 619 mg/L) with stable renal function. Post-treatment UPCE was also available in 5 cases with baseline dFLC (Freelite) <50 mg/L. In 2 of them the uMP was still visible but was no longer quantifiable, in 2 it remained stable and in one uMP increased from 20 to 40 mg/24h. UPCE can identify uMPs in patients with AL amyloidosis with a good sensitivity, and can quantify uMP excretion as low as 10 mg/24h. Changes in uMP excretion can be monitored during therapy, including patients with low FLC disease. Further studies are warranted to evaluate the response assessment.

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Hematology - Hemostasis

Cod: T224

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 leukemya 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.

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Hematology - Hemostasis

Cod: T225

PRELIMINARY EVALUATION OF THE 72 HOURS "BLAST/ABN LYMPH AND/OR ATYPICAL LYMPH" RULE DERIVED FROM THE RECOMMENDATIONS OF THE FRENCH-SPEAKING CELLULAR HEMATOLOGY GROUP (GFHC)

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Background
The GFHC recently published criteria for microscopic analysis of blood smears when a blood differential is requested. Cornet et al evaluated application of those criteria and proposed to suppress any leucocyte review during 72h in the context of remaining blast or atypical lymphocyte when no abnormal cells were found on the 1st slide involving the above flag at 0h and no new rule appeared in the course of a new blood differential. The aims of our study were to evaluate the risk of such cancelation and in a second time identify causes of sequence interruption.

Material & Methods
All the blood samples were collected in EDTA K3 Sarstedt® tubes and all the blood analysis were performed with XN-10 and XN-20 (Version 18) analyzers, SP-10 slide maker and a DM96 automated microscope (Cellavision®). The analyzers were also supplied with the Extended IPU (Version 3.1.7.) module, integrating manufacturer's recommendations and GFHC criteria. A retrospective study of 100 patients for whom the "72h rule" did at least apply once in a 7 days-period was undergone with a record of sequence of events.

Results
The 72h rule applied at 24h for 64 out of 73 patients (reduction rate of smear (RRS): 87.7%), at 48h for 41 out of 61 (RRS: 67.2%) and at 72h for 24 out of 48 (RRS: 50.0%). Further analysis allowed us to conclude that the rule applied two times in a row at 48h for 24 patients, at 72h for 12 patients and only for 2 patients three times consecutively. After concertation with Sysmex®, we identified the XN technical flag that often cancelled the 72h rule (a.o. Blast Atypical Lymph) and that might be transgressed to extent this rule application. During the period of our study, only 1 myeloma patient for which a smear was reviewed showed plasma cells. Noteworthy, those were already present at J0 but not reported on the initial smear. This highlights the importance of human expertise while reviewing smears. Indeed, a wrong application of this rule, due to an erroneous manual review, may be detrimental to the patient.

Conclusion
The 72h rule offers significant contribution to workflow optimization of the hematology laboratory processes and thus reduce the production cost and the turnaround time of blood differential results. Its effectiveness decreases over time especially due to XN technical flag changes (e.g. Blast Atypical Lymph). The implementation of this rule requires, at first, a careful examination of the smear avoiding initial false negative 72h rule results.

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Hematology - Hemostasis

Cod: T226

EVALUATION OF THE O-CONCEPT FROM SYSMEX® IN CLINICAL SETTINGS

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Introduction
Mean Cell Haemoglobin Concentration (MCHC) plays an important role in laboratory quality control while allowing detection of potential causes of erroneous results, such as hyperlipidaemia or haemolysis as well as RBC disease with true hyperdense cells. The evaluated "CBC-O App" in our laboratory applied to every patient with MCHC > 36,5 g/dl and Haemoglobin (Hb) < 10 g/dl or with MCHC> 37,5 g/dl. This app is working as a guide when approaching an unexplained increased MCHC.

Material and methods
The CBC-O App uses the comparison of RBC, HGB provided by impedance and photometry at room temperature to RBC-O and HGB-O delivered by flow cytometry measured at 41°C to classify them and offer efficient alternative solution as proposed by Berda and al.
On a 5-weeks period, 116 samples (36 patients) out of a total cohort of 27,048 (0,4%) were positively screened for the "CBC-O App" on the XN 9000. Blood smears were analysed on a DM96 CellaVisionTM and chemistry assays noticed for hypo-osmolarity and optical interference.

Results
The O-Concept rule applied for 116 of the samples: 106 required a control of the pre-analytical conditions, others reasons included turbidity (2), cold agglutinins (2), red cell-disease (6).
Suspected turbidity were confirmed by centrifugation. Only one true cold agglutinins and one red cells disease were found after blood smears assessment.
In order to control pre-analytical conditions, we reviewed our 106 sample's patients medical history. Immunosuppressive treatment or chemotherapy explained most of the cases (79%) while hyponatremia (8,5%) or combination of both previous conditions (5%) were found in the other cases. Only 7.5 % of cases remained unexplained.

Conclusion
Up to now and based on our experience, the "CBC-O App" is a useful tool for reporting the right RBC parameters in case of high MCHC.
The "CBC-O App" is efficient for detecting and managing high MCHC offering significant gain of time and a complete standardization of the process.

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Hematology - Hemostasis

Cod: T227

EPIDEMIOLOGY OF CRYOGLOBULINEMIA: A VIEW FROM THE WESTERN MEDITERRANEAN

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Background
Cryoglobulins are single or mixed immunoglobulins that undergo reversible precipitation at low temperatures. The presence of cryoglobulins in serum may result in a wide array of clinical syndromes of systemic inflammation caused immune complexes, or causing no syptoms at all. This is a rare disorder overall, although a number of diseases can be the origin of it. Systemic lupus erythematosus, lymphoproliferative disorders, some parasitic diseases or Hepatitis C virus infection are are known to cause cryoglobulinemia. The female-to-male ratio is 3:1 and mean age reported is 45-55 years.
Objetive
To revise the epidemiology of cryoglobulinemia among the population hosted by a provincial hospital in our country.
Material and methods
We collected all positive cryoglobulinemia test results performed from 2010 to 2016 by serum immunofixation and studied patients' clinical records in search for known causes of the disease according to bibliography for each individual. We also collected and processed their most relevant epidemiological data (age, gender and cryogloublin type).
Results
We studied eighteen patients who had received a positive result of cryoglobulinemia in serum immunofixation from 2010 to 2016, and revised their clinical records to discover the original pathology that had caused them. 78% were hepatitis C positive while the remaining cases belonged to systemic lupus eritematosus, visceral leishmaniasis, a non-Hodgkin lymphoma, and a inconclusive diagnosis. IgM k and IgML were the most frequent combination of immunoglobulines detected in immunofixation. The mean age of patients were 73 years-old, and the comparisson between genders unveiled a higher proportion of women 3:5.The prevalence of cryoglobulinemia in our area can be inferred as being 9:100000 for our hospital hosts a population area of approximately 200000.
Discussion
Prevalence of cryoglobulinemia due to HCV infecton in Mediterreanean areas vary from 80 to 90% of all mixed types, albeit cases in northern latitudes are more related to autoimmune disorders. Lymphomas and leukemias are oncological diseases whose link to cryoglobulinemia has been more successfully investigated. We paid special attention to the relationship between cryoglobulinemia and visceral leishmaniasis. Leishmania infection, which is a endemic disease in the Mediterreanean and Southern European area, may be an often misdiagnosed cause of cryoglobulinemia.

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Hematology - Hemostasis

Cod: T228

ASSESSMENT OF BIOLOGICAL RESPONSE TO DESMOPRESSIN USING VON WILLEBRAND FACTOR MULTIMERS ANALYSIS

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Background
Desmopressin (DDAVP) is a synthetic analog of vasopressin and used in the treatment of von Willebrand disease (VWD) and mild hemophilia A. A test dose of DDAVP is recommended to assess the biological response and to predict clinical efficacy during bleeding. The aim of this study was to assess the response of VWF:Ag, VWF:Ac, FVIII:C and the new commercially available VWF multimer assay to DDAVP administration.
Methods
Efficacy of DDAVP was investigated in 7 patients with bleeding tendency and family history of bleeding. Plasma VWF:Ag, VWF:Ac, FVIII:C and VWF multimers analysis was performed before, 1 hour after (peak), 2 hour and 4 hour after (clearance) the administration of DDAVP. Plasma VWF multimeric distribution was analyzed by agarose gel electrophoresis and immunofixation followed by densitometric analysis using new Hydragel 5 von Willebrand Multimers kit on the Hydrasys system (Sebia, France). Patients were classified according to response to DDAVP as complete responders, partial responders and nonresponders.
Results
6 patients, who did respond to desmopressin, had reduced VWF:Ag, normal VWF:Ac levels, VWF:Ac/ VWF:Ag $^{>}$0.7, normal ristocetin-induced platelet aggregation (RIPA) and normal multimeric structure of VWF. The baseline medians were as follows: VWF:Ag – 56.5 (IQR 47.3–66.5), VWF:Ac – 63.0 (IQR 61.0–70.5), FVIII:C – 98.5 (IQR 70.8–106.5). The medians of the peak point, 1h after DDAVP administration, were: VWF:Ag – 133.5 (IQR 87.3–187.3), VWF:Ac – 178.5 (IQR 133.8–234.0), FVIII:C – 302.0 (IQR 257.5–372.8). High-molecular-weight multimers (HMWM) slightly increased after DDAVP administration and this proportion did not change significantly during 4h. One patient was classified as type 2A VWD patient, demonstrating partial response to DDAVP: VWF:Ag 25% vs 67%, VWF:Ac 12% vs 36%, FVIII:C 41% vs 119%, VWF:Ac/ VWF:Ag 0.48 vs 0.54, reduced RIPA and a decreased fraction of HMWM.
Conclusion
New Sebia Hydragel 5 von Willebrand multimers test compared to difficult in-house methods is rapid (within-day results) and sensitive. It provides useful information not only for differentiating types/subtypes of VWD, but also for monitoring the response to therapeutic interventions such as DDAVP. In this study, all cases yielded the release of HMWM after DDAVP administration

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Hematology - Hemostasis

Cod: T229

RED BLOOD CELL ANTIBODY ASSAYS IN DARA-TREATED PATIENTS

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Background Daratumumab (DARA) is a treatment of multiple myeloma refractory to conventional chemotherapy. DARA is an experimental human monoclonal anti-CD38 IgG1κ: by binding with high affinity to tumor cells that express the CD38 signaling molecule on their surface, DARA destroys these plasma cells by various mechanisms. Through its anti-CD38 activity, DARA also recognizes red blood cells (RBC) as they weakly express the CD38 target molecule. This leads to turn positive RBC antibody screening and identification by indirect antiglobulin test (IAT) up to 6 months after the last injection of DARA. This interference may mask the presence of RBC alloantibodies at IAT.

Methods We describe the RBC screening and identification profiles of 29 patients treated with DARA, performed by IAT (solid-phase immunocapture technique and gel column filtration), enzymatic method (papain treatment) and dithiotreitol (DTT) treatment of panel cells.

Results All patients show a low intensity panagglutinin (1 + or 2+) at gel column filtration IAT. The reactivity observed at IAT performed by solid-phase immunocapture technique is more heterogeneous. Autocontrol and direct antiglobulin test are mostly negative (27 cases out of 29). All 29 profiles obtained after papain and DTT treatments are negative: extended phenotype matched transfusions may explain the absence of alloimmunization in these patients.

Conclusion A homogeneous and mostly low intensity panagglutinin at column filtration IAT is characteristic of DARA interference. As transfusions are often necessary in patients with myeloma, removing the interference is required to check possibly masked alloantibodies. For this purpose an enzymatic treatment (trypsin or papain) and a DTT treatment are necessary. The limitations of each treatment must be taken into account since some antigen systems may be damaged. Alloantibodies to KEL system cannot notably be excluded after papain and DTT treatments, therefore KEL1 antigen matched RBC transfusions are recommended in chronically transfused patients.

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THE USE OF CAPILLARY ELECTROPHORESIS FOR HEMOGLOBINOPATHIES DIAGNOSIS: PRELIMINARY ANALYTICAL PERFORMANCES OF CAPILLARYS 3 TERA, SEBIA

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Background: Hemoglobinopathies are the most common monogenic diseases in human. About 7% of world population is carrier and 2.7 conceptions per 1000 are affected by these pathologies. Hemoglobinopathies are qualitative and/or quantitative disorders of the hemoglobin. They can lead to several clinical symptoms, from benign (mild microcytosis) to the most severe (sickle cell disease, hydrops fetalis) with multiple end-organ damages. Diagnosis of hemoglobinopathies is mainly based on Complete Blood Count and on the separation and quantification of the different hemoglobin fractions, that can be done by HPLC, IEF or more recently, by Capillary Electrophoresis. Here we describe the analytical performances of the new hemoglobin diagnosis kit on CAPILLARYS 3 TERA, high-throughput and high-volume capillary electrophoresis instrument from SEBIA.

Methods: Resolution (Hb variants detection) of the method was assessed. Precision, limit of detection, linearity and carry-over studies were performed on CAPILLARYS 3 TERA with the dedicated CAPI 3 HEMOGLOBIN(E) kit, according to the CLSI EP05, EP06 and EP17 protocols. Interference with triglycerides and bilirubin were assessed (CLSI EP07 protocol). Correlation study with CAPILLARYS 2 Flex Piercing was carried out according the CLSI EP09 protocol.

Results: The CAPILLARYS 3 TERA is able to pick up all major common Hb variants (Hb S, F, C, D, E, G-Philadelphia, Constant Spring etc) with no interference with Hb A2 fraction. Total imprecision (including intra-, inter-assays, and inter-lots) is good for all Hb fractions considered (CV for Hb A, A2, S, C, D, E < 10%, CV for Hb F < 20%). A good linearity was observed for the same Hb fractions. No carry-over has been detected. No influence of triglycerides and bilirubin was noticed. The correlation between CAPILLARYS 3 TERA and CAPILLARYS 2 Flex Piercing is excellent ($r > 0.980$), showing perfect agreement in measuring Hb A, Hb A2, Hb S and major common Hb variants.

Conclusion: CAPILLARYS 3 TERA from SEBIA is a multiparametric capillary electrophoresis instrument, able to analyze whole blood at a high throughput. Separation and quantification of the hemoglobin fractions are accurate. Its good overall analytical performances make it an instrument of choice for high volume laboratories that would perform hemoglobinopathies diagnosis at high degree of confidence.

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HEMOSTASIS DISORDERS CORRECTION WITH PROTHROMBIN COMPLEX CONCENTRATE IN EARLY INFANTS AFTER CARDIAC SURGERY

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The excessive bleeding after cardiopulmonary bypass due to different factors is serious complication and leads to organ dysfunction and mortality increase. Congenital heart defects surgery correction in early infants (1-3 years old) with low weight has risk of bleeding due to hemodilution and blood coagulation factors deficiency (prothrombin complex, antithrombin III, protein C and S).
Purpose. To evaluate efficacy and safety of Prothrombin Complex Concentrate (PCC) using in early infants with congenital heart diseases based on clinical and laboratory research results.
Methods. PCC (Prothromplex 600) containing F II – 600 IU, F VII – 500 IU, F IX – 600 IU, F X – 600 IU, protein C – 400 IU, heparin – 300 IU, AT III – 15-30 IU was used in perioperative period in 15 patients aged from 9 months to 3 years, weighting 7.1-15.0 kg with congenital heart diseases after cardiopulmonary bypass. Blood loss, coagulation tests, coagulation factors levels, hematologic and biochemical parameters were evaluated. Prothromplex 600 single dose was 30-50 IU/kg. All data are expressed as median and interquartile range.
Results. Reduction in blood loss or blood loss cessation were registered in 13 (87%) patients. After PCC infusion INR decreased significantly from 1.5 (1.3;1.6) to 1.1 (1.0;1.5), factor VII activity increased from 43.8 (41.9;59.4)% to 105.7 (87.9;124.6)%, IX from 80.5 (77.9;83.2)% to 87.9 (61.5;113.6)%, X from 73.1 (55.1;91.9)% to 119.1 (111.6; 286.7)%, protein C activity increased from 66.0 (35.0;69.0)% to 100.0 (98.0;122.5)%, protein S from 56.3 (39.3;76.2)% to 96.8 (77.9;130.1)%, antithrombin III from 79.0 (76,0;80.7) to 86.5 (78.2;98.5)%.
Conclusion. PCC is an effective hemostatic agent which restores hemostasis system. PCC had no impact on hemodynamics. There were no thromboembolic events and allergic reactions. The dose of 30 IU/kg was enough to stop bleeding. The dose of 50 IU/kg used in massive hemorrhage was effective and safe.

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AFFINITY CHROMATOGRAPHY WITH DYE – NEW METHOD FOR THE PURIFICATION OF FACTOR VIII

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Background. Transfusion of blood is a medical therapy that can be life-saving. A lot of therapeutic products can be extracted from human blood.
Blood clotting is a sequential process of chemical reactions involving plasma proteins, phospholipids and calcium ions. Absence of any of these factor leads to bleeding disorders. The Factor VIII (FVIII) is one of the blood coagulation factor and it deficient causing development of bleeding desorders known as
Haemophilia A. Treatment of this disease are managed by plasma, cryoprecipitate or factor concentrate.
The aim of the work: optimization of a process for the extraction of human coagulation FVIII from Cryoprecipitate by the method of negative affinity chromatography.
Methods: ion-exchange chromatography on Poros® 10 SP (PerSeptive Biosystems), affinity chromatography on the Diasorb-aminopropyl matrix with Procion Blue HB as ligands.
Results: We investigated the parameters of a chromatographic process suitable for industrial scale to obtain a highly purified FVIII have been used increasingly in the last few years for plasma fractionation.
The Cryoprecipitate was initial raw material for the work. It was resuspended and purified with Al(OH)3 and PEG-4000. The mixture was processed by ion-exchange chromatography (the working buffer: 50 mM Tris-HCl, 1 mM CaCl2, 100 mM NaCl and 10 mM sodium citrate, pH 7,0). The next step was affinity chromatography of eluate FVIII.
Dye affinity chromatography is a protein purification procedure based on the high affinity of immobilized dyes for the binding sites on many proteins. There are three types of dye affinity chromatography: negative, positive and tandem chromatography. The principle of negative chromatography: the undesired proteins are retained by the immobilized dye while the desired proteins flow through the column. Chromatographic sorbents are using where the matrix was Diasorb-aminopropyl and Procion Blue HB as a ligand. As a result, the specific activity increased 110 times (from 0.087 IU/mg protein to 9.570 IU/mg protein).
Conclusions: We have high degree of purification FVIII as a consequence of additional procedures – affinity chromatography on the Diasorb-aminopropyl matrix with triazine active dyes Procion Blue HB as ligands.

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BROWNISH PLASMA COLOR, ASSOCIATED WITH ELTROMBOPAG ADMINISTRATION

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Background
Myelodysplasic syndromes (MDS) are clonal blood diseases characterized by abnormal differentiation and maturation of bone marrow cells, which usually cause pancytopenia. It exists an agonist drug of thrombopoietin receptor, indicated in case of severe chronic thrombopenia (TP) called eltrombopag (ET), with hepatic metabolism, faecal elimination and 21-32 hours of half life.

Case
Patient (P) 69 y.o., with type 2 diabetes mellitus and no drug allergies known to date. Diagnosed MDS 5q-. Treated with lenalidomide (2 years) and with azacytidine (2 years) with loss of response to both drugs. The patient presented severe TP (ST) (<10,000 platelets/μL) with refractoriness to platelet (Pt) transfusions. Due to ST with high risk of bleeding, eltrombopag treatment was initiated with progressive increase from 50 to 150 mg/day.
The P came to the Emergency Department two weeks after starting 150 mg/day dose, presenting a stalk tint (jaundice does not appear). Requested analytical: haemoglobin (Hb) 9.6 g/dL, Pt: 11000/μL, leukocytes (L): 2400/μL, GPT: 74 IU/L, GGT: 196 IU/L, ALP: 199 IU/L, ferritin:1934 μg/L, LDH: 1240 IU/L (240-480 IU/L) and direct Coombs doubtful. The plasma had brownish coloration (color as cola) with icteric index (II): 3.04 mg/dL (0-1.6 mg/dL), the total bilirubin (B) 0.62 mg/dL, and direct B 0.27 mg/dL, (both normal). Methemoglobinemia or porphyria was susspected. Methemoglobin fraction of 0.2% was normal, and porphyria was discarded for family history.
ET was suspended temporarily, the P was admitted and discharged the next day. After a week the P was checked, Hb 8.3 g/dL, Pt: 8,000/μL L: 3,300/μL, GPT: 73 IU/L, GGT: 203 IU/L, LDH: 1,408 IU/L and direct Coombs negative. The plasma had normal color, with a II of 1.58 mg/dL

Discussion
The use of new treatments creates a situation of uncertainty about possible interference (I) that may occur with the usual analytical methods in the laboratory. In this case, detecting the plasma color, first thing evaluated was the possibility of an adverse reaction. In the literature, color changes are described in plasma at higher doses, but not associated with any adverse effects. It is necessary to evaluate possible I by colorimetric analytical techniques. The most likely would B I. It has not been described I to 150 mg/day with diazo colorimetric methods. The P after suspension of ET has presented normalization of plasma color and has maintained the same levels of total B, ruled out the possibility of I.

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WARFARIN MONITORING AMONG THE ELDERLY. DO RECOMMENDATIONS AND ELECTRONIC ALERTS AFFECT PRACTICE?

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Introduction: Elderly warfarin treated patients are prone to complications, and high quality monitoring is essential. The aim of this study was to assess the quality of warfarin monitoring in a routine situation, and in a situation with an antibiotic-warfarin interaction, before and after receiving an electronic alert.

Materials and methods: The study was a cross-sectional descriptive study among nursing home physicians, using web-based case histories. Case A represented a patient on stable warfarin treatment, but with a substantial INR increase within the therapeutic interval. Case B represented a more challenging patient with trimethoprim sulfamethoxazole (TMS) treatment due to pyelonephritis. In both cases, the physicians were asked to state the next warfarin dose and the INR recall interval. In case B, the physicians could change their suggestions after receiving an electronic alert on the TMS-warfarin interaction.

Results: 398 physicians responded. Suggested INR recall intervals and warfarin doses varied substantially in both cases. In case A, 61% gave acceptable answers according to published recommendations, while only 9% did so for case B. Among those with originally unacceptable answers for case B, 51% did not reduce the warfarin dose as recommended in the electronic alert. Having an INR instrument in the nursing home was associated with shortened INR recall times.

Conclusions: Practical advice on handling of warfarin treatment and drug interactions are needed. Electronic alerts, as presented in electronic medical records on warfarin-antibiotic interaction, were insufficient to change practice in 51% of physicians. Availability of INR instruments may be important regarding recall time.

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Hematology - Hemostasis

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THE ROLE OF CELL POPULATION DATA IN THE EARLY DETECTION OF SEPSIS

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Background: the parameters cell population data CPD reported by the analyzer XN (Sysmex, Kobe, Japan) reflect size and internal structure of leukocytes. We aimed to assess the clinical utility of the CPD as biomarkers for the early diagnosis of sepsis.
Materials and methods: the retrospective study included 995 patients split into two groups. The study group (G1) included 586 controls, with no quantitative nor morphological alterations in the hemogram, and 137 patients diagnosed with sepsis. Applying univariate logistic regression, optimal cut off for the diagnosis of sepsis and odds ratio (OR) for CPD were established. After that a multivariate logistic regression model was developed. OR and area under curve (AUC) were recorded. The test of Hosmer-Lemeshow was used to verify the goodness of fit of the final models.
A risk-stratification scale for diagnosing sepsis was developed considering the coefficients of the final multivariate model (Table).
The performance of the scale was studied in a validation group (G2); the inclusion criteria was the same as for the study group, 212 controls and 60 septic patients.
Results: the multivariate analysis in the G1 showed that the categorized variables Mono% NE WZ, MO WZ, LY WZ, MO X and NE WY were statistically significant (AUC >0.80, good calibration p = 0,237).
Using the above mentioned predictors, a score was composed (NEMO). NEMO stratified into three risk groups: mild(≤3), moderate (4≤NEMO≤9) and high (≥10).
When applied to G2 NEMO ≥10 obtained sensitivity 85.0%, specificity 98.5%, Positive predictive value = 94.4% and Negative predictive value 95.8%.
Conclusions: The prognosis of patients with sepsis relies on an early diagnosis to start the appropriate therapeutic.
CPD are reported as research parameters in routine analysis; NEMO score can be determined without extra blood sampling and low cost, yielding results within 15 minutes. It is potentially useful in the decision making for the early diagnosis of sepsis.

	Beta	OR (%95)	P	Weight	NEMO score
MO X ≥ 119	2.18	8.85 (4.88 – 16.03)	< 0.0001	6	
NE SFL ≥ 52	1.92	6.79 (3.72-12.42)	< 0.0001	5	
Mono ≤ 6.4	1.95	7.03 (3.86 – 12.79)	< 0.0001	5	
LY WX ≥ 546	1.56	4.77 (2.40 – 9.47)	< 0.0001	4	
LY Z ≥ 59	1.26	3.51 (1.91 – 6.48)	< 0.0001	3	
NE WX ≥ 327	0.77	2.17 (1.13 – 4.17)	< 0.0001	2	

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CLINICAL VALUE OF RETICULOCYTE HB CONTENT (MCHR) IN THE ASSESSMENT OF IRON REQUIREMENTS IN PATIENTS WITH INFLAMMATORY BOWEL DISEASE

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Background Anemia is significant and costly complication of inflammatory bowel disease (IBD). Anemia is multifactorial in nature, the most prevalent etiological forms being iron deficiency anemia (IDA) and anemia of chronic disease (ACD). Oral iron supplementation has been linked to extensive gastrointestinal side effects and disease exacerbation; however, many physicians are reluctant to administer iron intravenously, because in a condition associated with inflammation, such as IBD, the determination of iron status using common biochemical parameters alone is inadequate.
We investigated the reliability of reticulocyte hemoglobin content (MCHr) reported by CELL-DYN Sapphire analyzer in the assessment of the erythropoiesis status in IBD.
Methods: 124 outpatients with IBD were recruited. Serum iron, transferrin and ferritin were assayed in a Cobas c 711 (Roche Diagnostics, Manheim, Germany). Hemograms were performed on a CELL DYN Sapphire analyzer (Abbott Diagnostics, Santa Clara, CA, USA) within 6 hours of blood collection.
The differences between groups were evaluated using Student t-test, $P<0.05$ was considered significant.
Receiver operating characteristic (ROC) curve analysis was utilized, to assess the diagnostic performance of MCHr for detecting iron deficient erythropoiesis.
Gold standard for iron availability was transferrin saturation (TSAT) < 20 %, the test traditionally used to assess iron availability.
Cohen´s kappa test was calculated to verify the agreement between TSAT and MCHr.
Results: 53.5 % of the IBD patients had IDA, while 9.5 % had ACD and 37% had normal iron status.
The results in the IDA group reflected the state of iron depletion (low ferritin), low iron availability (low TSAT) and iron-restricted erythropoiesis (low MCHr); the ACD group suffered functional iron deficiency (normal or high ferritin, low MCHr).Significant differences in MCHr were found in those groups and normal group, $P<0.001$.
ROC Area under curve was 0.878 (95%CI 0.699-0.954), at a cut off 31.5 pg resulting in sensitivity 76.8%, specificity 100%.
Good agreement between TSAT and MCHr was found Kappa = 0.69.
Conclusions: MCHr provides information on iron availability in IBD patients. It is a reliable test to assess iron supply for erythropoiesis, and thus a good aid to guide iron therapy.

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FREE KAPPA LIGHT CHAIN AND IMMUNOGLOBULIN D KAPPA MULTIPLE MYELOMA. A CASE REPORT

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Background
Immunoglobulin D multiple myeloma is rare and has a poorer prognosis than other multiple myeloma isotypes. The presented case report is a free kappa light chain and kappa IgD multiple myeloma.
Case Report
A 65-year-old male presented to our hospital with suspected anaemia and lumbar pain over six months. Steroid injections in the back and oral calcium were performed during this time to combact the pain. After this treatment he improved slightly, but in the last two months he lost about 17 kg and had joint pain in knees and ankles. He presented intense nocturia every 2 hours 15 days prior to his visit to the hospital.
On physical examination, his vitals were normal. On laboratory investigation, hemoglobin was 11.4 g/dL (RV: 13.0-17.50 g/dL); his serum creatinine was 2.10 mg/dL (RV: 0.33-1.13 mg/dL), total proteins were 6.1 g/dL (RV: 6.4 -8.2 mg/dL) and albumin was 3.4 g/dL (RV: 3.4-5.0 g/dL). He had severe hypercalcemia (albumin corrected calcium: 15.4 mg/dL (RV: 8.5-10.1 mg/dL)). Tumoral markers were requested to exclude prostate cancer with bone metastasis, but all of them were normal. A proteinogram was performed and a monoclonal component was observed in gamma region. The results of serum immunofixation showed IgD # monoclonal light chain band and free # monoclonal light chain. Medicine laboratory specialist added the following tests: IgG 322 mg/dL (RV: 700-1600 mg/dL), IgA 37 mg/dL (RV: 68-379 mg/dL) and IgM 9.23 mg/dL (RV: 41,0-251 mg/dL). Serum free # chain level was elevated (2860 mg/L (VR: 3,3-19,4 mg/L)) as well as elevated free κ/λ ratio (1513.23). Urine immunofixation presented Bence-Jones proteinuria (free # monoclonal light chain band). Bone marrow biopsy showed 18% of plasma cells and patient was found to have lytic lesions in some vertebras.
All this data was concordant with the diagnosis of IgD λ monoclonal light chain producing plasma cell myeloma in International Staging for Multiple Myeloma II. His chemotherapy consisted of thalidomide/dexamethasone and cyclophosphamide. The patient achieved hematological remission of myeloma 6 months after the beginning of the treatment.
Conclusion
All multiple myeloma patients with light chain proteinuria and small or absent M-spike should be evaluated for IgD multiple myeloma isotype.

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CLINICAL INTEREST OF SAME-DAY RESULTS ASSAY "HYDRAGEL 5 VON WILLEBRAND MULTIMERS" FOR THERAPEUTIC DECISION

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BACKGROUD

Bleeding disorders such as inherited-type 2 and acquired von Willebrand Disease (vWD) are related to defects in von Willebrand factor (vWF) multimers' distribution. Up to present, home-brew electrophoretic test is the only technique that allows detecting defects in VWF multimers' distribution. It is a time-consuming technique, difficult to standardize, available only in specialized labs and with a long time to results (> 4 days). Therefore it is not adapted to guide immediate therapeutic actions. Sebia has developed a simplified multimers test that produces same day results. Here, we present three cases showing the clinical interest of this new test.

METHODS

Citrated plasma samples were analysed on the Hydrasys 2 instrument (Sebia, Lisses France) with a ready to use SDS agarose gel (Hydragel 5 von Willebrand multimers, Sebia). Multimers were visualized directly on the gel (w/o protein transfer), using an immunofixation with antibodies conjugated to horseradish peroxidase/ TTF1/TTF2. Curves were produced using the manufacturer's gel scanner and interpretation software.

RESULTS

For a woman with a suspected acquired vWD [vWF:Activity (Act) = 28 %, vWF:Antigen (Ag) = 49 %, ratio Act/Ag= 0.57 in basal conditions; vWF:Act = 58 %, vWF:Ag = 101 %;, ratio 0.58 after vWF concentrate infusion], the absence of high molecular weight multimers (HMWM) in both samples strengthened the hypothesis of an inhibitor against vWD, because of an accelerated turn-over of HMWM of vWF concentrates.

Analysis of vWF multimers of a pregnant woman with a moderate bleeding tendency (mainly bruises), a prolonged bleeding time (occlusion time quantified by PFA-EPI assay > 300 sec) and abnormal vWF:Act/Ag ratio (vWF:Act = 68%, vWF:Ag = 228 %, ratio = 0.3) indicated an excess of unpolymerized vWF, but this patient kept a part of HMWM and no treatment was necessary for the delivery. For a third patient with unexplained hemorrhagic syndrome (vWF:Act = 32 %, vWF:Ag = 88 %; ratio = 0.36) non responsive to desmopressin (vWF:Act = 77 %, vWF:Ag = 131 %) the multimer analysis showed an absence of HMWM.

CONCLUSION

This new assay is a valuable tool for the diagnosis of constitutive or acquired VWD as well as an evaluation of the hemorragic risk. It provides clear pattern of VWF multimer distribution and has the major advantage to be performed within day. The clinical cases here presented show the importance of multimer analysis in therapeutic decision making

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INTERCHANGEABILITY OF CD34+ CELLS QUANTIFICATION FOR CLINICAL PURPOSE

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Background. Transplantation of hematopoietic progenitor cells is being used in the treatment of blood disorders, malignancies, and genetic abnormalities. The CD34 antigen is found to be present on immature hematopoietic precursor and progenitor cells, and serves as useful marker to quantify them. Nowadays, flow cytometry is a method of choice in enumeration of CD34+cells. Aim of this study was performance evaluation of BD Stem Cell Enumeration clinical software on BD FACS Canto II flow cytometer using BD Stem Cell Enumeration kit, as well as interchangeability of results for clinical purpose.
Methods. CD34+ cell quantification protocol for BD flow cytometer was verified in the medical biochemistry laboratory accredited according to ISO 15189 norm following CLSI EP-A2 protocol. Verification studies included assessment of random error check upon triplicate measurement for five days consecutively and calculation of measurement uncertainty. Comparison study was performed on 53 blood and external quality control samples on BD FACS Canto II and Beckman Coulter Cytomics FC500 flow cytometers. The calculations were made using Statis Pro and MedCalc software.
Results. Verification studies of Stem Cell Enumeration clinical software on BD FACS Canto II flow cytometer fulfil quality specification. Within laboratory precision was 5.009 and 5.497%, respectively, for commercial control samples at low ($10.5x10^6$/L) and high concentration level ($31.7x10^6$/L). Expanded measurement uncertainty was 15.6 and 15.2% for the same controls. Passing and Bablok regression analysis for 53 samples revealed satisfactory comparison (Y=-0.3067 +0.9933X; R=0.9907). Additionally, the obtained results were evaluated through participation in CD34 Stem cell enumeration, an external quality assessment scheme, organized by UK NEQAS for Leukocyte Immunophenotyping, where results were within target values with insignificant bias from mean.
Conclusion. Verification studies of BD Stem Cell Enumeration clinical software on BD FACS Canto II flow cytometer using BD Stem Cell Enumeration kit fulfil analytical specifications suggested by manufacturer. Comparison study revealed possible interchangeability of the obtained results for clinical purpose.