Haematology, including haemostasis

W007

CLINICAL AND PARACLINICAL ASPECTS OF MONOCLONAL GAMMOPATHIES IN THE SOUSS-MASSA REGION IN MOROCCO: A RETROSPECTIVE STUDY .

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BACKGROUND-AIM

-The main objective of our work is to describe the epidemiological, clinical and especially biochemical profiles of monoclonal gammopathies in the Souss-Massa region in southern Morocco over a period of more than 10 years.

METHODS

- This is a retrospective study, we have exploited the records of patients diagnosed with monoclonal gammopathy at this center over a period of 10 years, between 2010 and 2020.

RESULTS

-117 patients were included in the study with a strong male predominance, 76 (65%) patients were male and 41 (35%) were female, with a male/female sex ratio of 1.85. The mean age± SD of our population was 61.44±14.54. monoclonal gammopathies in patients under 40 years of age represents a 6.8% of all gammopathies. The main symptoms of monoclonal gammopathy were as follows, bone pain in 66.6% (n=78), alteration of the general state with a rate of 47.8% (n=56), bone marrow compression syndrome12.8% (n= 15). anemia in 70.9% (n=83), renal failure in 40.17% (n=47) and hypercalcemia in 23.9% (n=28). In our study the following diagnoses were retained, multiple myeloma 82.05% (n=96), solitary plasmacytoma 8.5% (n=10), monoclonal gammapathies of undetermined significance 2. 60% (n=3), three cases of lymphoma 2.56% (n=3), two cases of plasma cell leukemia 1.7% (n=2) with a mean age of 64 years, two cases of Waldenström disease 1.7% (n=2) and one case of chronic lymphocytic leukemia. Also, the isotype distribution was as follows: IgG kappa 33.73% (n=28), IgG Lambda 21.68% (n=18), IgA Kappa 12.05% (n=10), IgA Lambda 7.22% (n=6), IgM kappa 3.61% (n=3) and IgD Lambda 2.41% (n=2). The biclonal peak was found in two cases with a percentage of 2.41%.

CONCLUSIONS

-A prospective study associated with an etiological study of the cases of monoclonal gammopathies is necessary, especially since our region is an agricultural region.

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W008

THE ADDED VALUE OF CELL POPULATION DATA IN THE DIAGNOSIS OF EXACERBATIONS OF COPD

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BACKGROUND-AIM

Chronic obstructive pulmonary disease (COPD) is characterized by pulmonary and systemic inflammatory processes; exacerbations are episodes of increased respiratory symptoms particularly dysnea, cough and sputum. There is no generally agreed definition for an exacerbation of COPD, since the variety of causes and severities, but a misdiagnosis can hamper the correct management.
Morphometric parameters (CPD), reported as part of leukocyte differentials by Sysmex XN analyzer, give information (medians and distribution width) of the size), nucleic acid content and internal structure of neutrophils, lymphocytes and monocytes.
We aimed to evaluate the value of CPD for the early diagnosis of patients with exacerbation of COPD.

METHODS

80 patients were recruited in the stable phase of the disease; CBC, markers of inflammation C reactive protein (CRP) and neutrophil/lymphocyte ratio (NLR) were analyzed and compared with the values obtained when the patients came to the Emergency Department with worsening conditions suggestive of exacerbation.
Mann–Whitney U-test was applied to detect statistical differences between stable COPD and exacerbation; $P < 0.05$ was considered statistically significant.
Receiver operating characteristic (ROC) curve analysis was used to establish the diagnostic performance of CPDs in the detection of an exacerbation of COPD.

RESULTS

CRP, NLR and NEUT-WY were significantly higher in patients with exacerbation p<0.001.
CPR 1.1 mg/L (0.8-2.7 mg/L) 8.8 mg/L (3.8-9.7 mg/L)
NLR 1.8 (1.3-2.3) 5.8 (1.7-7.8)
NEUT WY 611 au (597-628 au) 655 au (627- 694 au)
NEUT-WY was the CPD with the best performance for defining exacerbations.
Area under curve 0.832 (Confidence Interval 95 % 0.758 -0. 894) cut off >644 sensitivity 68.5% specificity 91.8%.

CONCLUSIONS

The correct and early identification of COPD exacerbations is vital, for therapy success and impact on patients' morbidity, mortality and quality of life.
CPD provide quantitative information of morphological and functional characteristics of leukocytes. NEUT-WY quantifies the increase in nucleic acid content in a heterogeneous population of activated neutrophils, which reflects the involvement of the innate immunity in the pathophysiology of the exacerbation.

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W009

LEUKOCYTE MORPHOMETRIC PARAMETERS BY SYSMEX XN ANALYZER IN THE DETECTION OF SARS-COV-2 PNEUMONIA

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BACKGROUND-AIM

Leukocyte differential, present certain features in SARS-CoV2 infected patients neutrophilia , lymphopenia and morphology alterations, which could be useful for screening.
Cell population data (CPD) are reported as part of leukocyte differentials by Sysmex XN analyze; they are morphometric parameters that characterize neutrophils, lymphocytes and monocytes and classify them according to their volume, granularity and their content in nucleic acids. CPD reflects in numbers the changes in morphology and activation status triggered by infections. We aimed to evaluate the predictive power of CPDs for the differential diagnosis of COVID19 versus non-COVID19 pneumonia.

METHODS

The prospective, observational, multicenter study was conducted in 3 hospitals, including patients > 18 years admitted with the diagnosis community-acquired pneumonia in the period November 2019 - October 2020.
Complete blood count were analyzed using Sysmex XN counters. Diagnosis of SARS-CoV-2 infection was done using real-time reverse transcription-polymerase chain reaction.
Patients were divided into two groups: (1) referral cohort in a hospital for the development of the model (2) the sample of two other hospitals for its validation.
Multivariate logistic regression model has been developed for the detection of COVID patients. Robustness of the model has been evaluated by means of the area under the ROC curve and the calibration of the model. Statistical significance $p < 0.05$.

RESULTS

598 patients were recruited, 322 in the referral cohort and 276 in the validation group.
The average age was 67.0 years (Standard Deviation 14.59 years) and 61.49 % male.
Neutrophil lymphocyte ratio, NE-WZ, LY-Y , LY-Z, LY-WX, LY- WY, MO- WY, MO-Z were included in the multivariate analysis, and presented a significant association for the differential diagnosis of SARS-CoV-2 infection with AUC of 0.84 (95% CI 0.82-0.92) in the referral cohort and 0.77 (0.69-0.85) in the validation cohort.

CONCLUSIONS

Leukocyte differential and CPDs could be very useful in the differential diagnosis of SARS-CoV-2 pneumonia and lead to a cheap and early diagnosis of the disease.

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W010

MACHINE LEARNING BASED DECIPHERMENT OF CELL POPULATION DATA: A PROMISING HOSPITAL FRONT-DOOR SCREENING TOOL FOR COVID-19

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BACKGROUND-AIM

Key challenges against early diagnosis of COVID-19 are its symptoms sharing nature and prolong SARS-CoV-2 PCR turnaround time. Hither machine learning (ML) tools experienced by routinely generated clinical data; potentially grant early prediction.

METHODS

Routine and earlier diagnostic data along demographic information were extracted for total of 21,672 subsequent presentations. Along conventional statistics, multilayer perceptron (MLP) and radial basis function (RBF) were applied to predict COVID-19 from pre-pandemic control. Three feature sets were prepared, and performance evaluated through stratified 10-fold cross validation. With differing predominance of COVID-19, multiple test sets were created and predictive efficiency was evaluated to simulate real-fashion performance against fluctuating course of pandemic. Models validation was also inducted in prospective manner on independent dataset, equating framework forecasting to conclusions from PCR.

RESULTS

RBF model attained superior cross entropy error 20.761(7.883) and 20.782(3.991) for Q-Flags and Routine Items respectively while MLP outperformed for cell population data (CPD) parameters with value of 6.968(1.259) for 'training(testing)'. Our CPD driven MLP framework in challenge of lower (<5%) COVID-19 predominance affords greater negative predictive values (NPV >99%). Higher accuracy (%correct 92.5) was offered during prospective validation using independent dataset. Sensitivity analysis advances illusive accuracy (%correct 94.1) and NPV (96.9%). LY-WZ, Blasts/Abn Lympho?, 'HGB Interf?', and 'RBC Agglutination?' are among novel enlightening study attributes.

CONCLUSIONS

CPD driven ML tools offer efficient screening of COVID-19 patients at presentation to hospital to backing early expulsion and directing patients' flow-from amid the initial presentation to hospital.

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W011

USING CALCULATED GLOBULIN VALUES BELOW THE REFERENCE RANGE AS A SCREENING TOOL FOR HYPOGAMMAGLOBULINEMIA AND LIGHT CHAIN MYELOMA

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BACKGROUND-AIM

Both primary immune deficiency (common variable immunedeficiency,CVID) and light chain myeloma require medical intervention and diagnostic delay can exacerbate morbidity. This study investigated the potential use of reflex testing at low levels of calculated globulin to detect antibody deficiency and light chain myeloma

METHODS

Serum samples from community physicians and outpatient clinics for liver functions test with low calculated globulin (<16 g/L RR 18-37 g/L) levels were reflex tested for immunoglobulins and protein electrophoresis. In addition patients with globulin levels <16 g/L with identified measured immunoglobulin levels and protein electrophoresis were included in the analysis.

RESULTS

The study, carried out from January 2019 to December 2019 included 139 patients who were investigated by reflex testing at low globulin values and 110 patients with globulin values <16 g/L with protein electrophoresis and immunoglobulin data. Analysis of the clinical diagnosis associated with low globulin levels showed: haematological malignancy n=116, age 34-88y, IgG <3-7.6 g/L; immunosuppressant therapy n=35, age 36-88 y, IgG<3 -7.6 g/L, antiepileptic treatment n=11, age 33-83y, IgG<3-4.7 g/L, antiepileptic and immunosuppressant treatment n=4, age 57-82y, IgG<3-5.5 g/L, CVID n=4, age 38-61y IgG<3 -4.0 g/L, light chain myeloma n=20, age41-87y, IgG<3-6.7 g/L, non-hematological malignancy n=2, age 82 and 87, IgG<3-3.1g/L, non-specific diagnosis n=57, age 33-97y, IgG<3-8.5g/L. Three of the 4 patients with CVID were diagnosed by reflex testing in the last 8 years prior to the study. 6 patients with light chain myeloma were diagnosed by reflex testing in the last 12 years: 3 light chain monoclonal gammopathy of undefined significance and 2light chain myeloma. A single patient with abnormal light chain ratio, and severe back pain diagnosed during the study, died prior to follow up. Of the 57 patients with non specific diagnosis: 11 transient decrease in globulins, 11 referred to either hematology/immunology specialities, a further 3 with normal and 5 with subnormal functional antibody tests and 27 had several other co-morbidities. Among the latter, 86 year old male with IgG of 3.5g/L presented with sepsis, with a recurrence of thymoma

CONCLUSIONS

The study suggests reflex screening at low globulins levels detects CVID and light chain myeloma patients. Optimising treatment in patients with other clinical co-morbidities may require careful clinical and laboratory assessment and close monitoring

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W012

LEARN FROM YESTERDAY, ACT TODAY AND REMEMBER TOMORROW! – CASE-REPORT AND SHORT REVIEW OF DABIGATRAN OVERDOSE

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BACKGROUND-AIM

Oral Dabigatran Etexilate is an anticoagulant prodrug whose active form acts as a direct thrombin inhibitor agent. Dabigatran plasma concentration has its peak 1-3h after intake and its half-life is 12-24h. Dabigatran's antidote – idarucizumab – binds dabigatran with higher affinity than thrombin.
The authors present a case report.

METHODS

23-year-old woman was admitted in the Emergency Room (ER) 6h after deliberately ingesting 3000mg acetylsalicylic acid, unknown dose (1125mg or 1650mg) of dabigatran. She denied ingesting any psychotropic substances and having gastric complaints. The patient has a history of overdose and unknown psychiatric disease. At clinical observation, she was hemodynamically stable with no signs of active haemorrhage. A gastric lavage was performed. The patient was hospitalized for monitoring and possible administration of the antidote. Initial blood tests were within reference values, except the prothrombin time (PT) 24.4s and activated partial thromboplastin time (aPTT) 70.7s.

RESULTS

12 hours after drug ingestion, the ER asked the emergency laboratory (EL) for help to monitor the patient's bleeding risk. The EL suggested measuring Dabigatran level/Diluted TT (dTT) (380.2ng/mL), thrombin time (TT) (immeasurable) and aPTT (47.4s). Once dTT was above 200ng/mL, we were facing a high haemorrhagic risk: "should the antidote be used in a young and hemodynamically stable patient with no signs of bleeding?". The decision was to administer idarucizumab 5g. The following coagulation tests were measured 13h after the antidote intake: aPTT 22.6s and dTT<20ng/mL.

CONCLUSIONS

aPTT plays a major role as a qualitative predictor when handling a possible Dabigatran overdose and its haemorrhagic risk. Yet, TT is unsuitable in emergency situations, as it is immeasurable in face of high levels of Dabigatran plasma concentration. In our case, despite a late laboratorial monitoring and decision taking, in the end all went well with our young patient with no comorbidities. This made us review Dabigatran's pharmacokinetics and the correct use of the different coagulation tests. We found it very important to be acquainted with these rarely used but relevant guidelines. A multidisciplinary approach makes a difference and saves lives.
Disclosure: No conflicts of interest.

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W013

CAN WE SOLVE INTERFERENCE IN THE WBC DIFFERENTIAL COUNT?

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BACKGROUND-AIM

Abnormal white blood cell (WBC) scattergrams can lead to incorrect five-parameter differential count (DC) leading to manual leucocyte differential by microscope observation of blood smears. Here, we aimed at evaluating bilirubin (BR) and target cells (TC) as possible causes of DC interference and analyze sample dilution on the latter.

METHODS

We selected 74 samples with abnormal WBC scattergrams and DC interference (Beckman Coulter DXH900). Diluted DC was obtained using a 1:3 dilution (Beckman Coulter's DxH Diluent). Manual DC was performed by microscope observation of blood smears with Wright-Giemsa staining. The differences between percentages of Neutrophils (NE) and Lymphocytes (LY) in predilution (PRD), postdilution (PSD) and manual count (MC) were established. Significance of these differences was evaluated by Wilcoxon test. Spearman's test was used to calculate the correlation between BR, NE and LY. BR interference was evaluated using 1:3 dilution of normal WBC scattergram samples with hyperbilirubinemia (>5 mg/dL) serum samples. Analysis of TC interference was assessed by microscope observation of samples with abnormal scattergrams, no DC interference and normal BR serum levels.

RESULTS

Dilution solved 100% of DC interference. Wilcoxon test evidenced no differences among PRD vs PSD and PSD vs MC for NE (p=0.99 and p=0.56, respectively). Regarding LY, Wilcoxon showed differences between PRD vs PSD (p=1.79x10-13), but not between PSD vs MC (p=0.8).
Hyperbilirubinemia and TC were present in 70% and 95% of samples, respectively. We obtained a moderate positive correlation (r=0.412) between BR and NE (PRD-PSD) and a weak positive correlation (r=0.328) between BR and LY (PRD-PSD). The samples evaluated for BR interference showed no evidence of DC interference or debris in scattergrams. Microscopic slides observed for TC interference presented at least 5 TC per field and debris in WBC scattergrams.

CONCLUSIONS

Our results suggest that DC interference and abnormal WBC scattergrams are more related to TC than to BR. The fact dilution solved the observed interference seems to indicate that lysing reagent is unable to effective destroy TC. The dilution lowers erythrocyte counts, favoring destruction by lyses reagent. These findings bring the opportunity to introduce reflexive diluted samples.

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W014

LEUCOERYTHROBLASTIC SYNDROME. IMPORTANCE OF CORRECT DIFFERENTIAL DIAGNOSIS

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BACKGROUND-AIM

Leucoerythroblastic syndrome or leucoerythroblastosis is the presence in peripheral blood of nucleated erythroid and immature myeloid cells. It is due to the occupation of bone marrow by not hematopoietic cells (myelophthysis). Its causes may be hematological or not. The most prevalent cause is bone dissemination of solid tumors (mainly breast and prostate tumors), accounting for 40% of the cases. Among hematological causes, idiopathic myelofibrosis is the most prevalent.

METHODS

We expose here a case of a 41 year old woman with chronic lumbalgia. Latelly, the patient refers an exacerbation of pain which does not remit with analgesia and is supposing a functional limitation. In order to determine the etiology a nuclear magnetic resonance (NMR) is performed. It shows an alteration in red bone marrow for which the patient is derived to the Hematology Unit.

RESULTS

A complete blood analysis is performed which shows a microcytic hypochromic anemia of 7.3 g/dL of hemoglobin with the presence of 4% of erythroblasts and 11% of immature granulocytes. The peripheral blood smear shows morphologically conserved granulocytic cells with intense myelemia. Blasts are not seen. Concerning to red blood cells, they show anisocytosis, microcytosis, polychromasia, poikilocytes, elliptocytes and occasional dacryocytes. 4 erythroblasts are found in 100 leukocytes counted.
These findings orient the diagnostic suspicion to an idiopathic myelofibrosis. In consequence, a molecular study (BCR-ABL, JAK2, CALR and MPL genes), a computed axial tomography (CAT) and a bone marrow biopsy are requested.
In the CAT, a mass in the left breast is observed, suggestive of being a neoplasia. In addition, tumor spread into ganglia, liver and bone marrow is visualized. The molecular study and the bone marrow biopsy are cancelled. The leucoerythroblastic syndrome observed in the patient is associated with this tumor spread into bone marrow and a possible hematological cause is dismissed.

CONCLUSIONS

The case exposed shows how some non-hematological pathologies as bone infiltration of solid neoplasia can mimic analytical characteristics of hematological pathologies. In these cases is especially important to establish a correct differential diagnosis in order to set up the treatment most appropriate to the patient´s situation.

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W015

ELTROMBOPAG INTERFERENCES STUDY IN CREATININE AND BILIRUBIN SERUM MEASUREMENTS

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BACKGROUND-AIM

Eltrombopag is a thrombopoietin receptor agonist used in the treatment of aplastic anemia. In solution, the color of Eltrombopag depends on its concentration and pH, due to that coloration with an absorbance peak between 450-550 nm, it has the potential to interfere with some laboratory tests. The data sheet states interference with total bilirubin and creatinine. Fifteen percent of patients with this treatment may develop severe hepatotoxicity and bilirubin levels should be monitored during treatment. The aim is to identify and study the interference with bilirubin and creatinine, for the correct interpretation of its results in the monitoring of these patients.

METHODS

Experimental study conducted in July 2021. Serum containing metabolites of a 150mg/24 hour regimen of Eltrombopag was used. Six dilutions 1:15, 1:6, 1:3, 1:2, 3:1 and 6:1 (serum:interferent) were performed to evaluate the degree of interference in creatinine, total and direct bilirubin [Alinity ci series, Abbott Diagnostics,US], considering other interferents (jaundice, lipemia and hemolysis). The following concentration ranges were studied: creatinine [0.67-5.75 mg/dL], total bilirubin [0.79-7.62 mg/dL] and direct bilirubin [0.32-4.30 mg/dL]. The result was considered interfered when the percentage difference between the estimated and measured values was greater than 1.64 times the analytical coefficient of variation (creatinine 7.38%, total bilirubin 14.68% and direct bilirubin 15.10%).

RESULTS

Positive interference was observed in creatinine from values higher than 0.95 mg/dL in a range of 7.98 to 22.06%. Negative interference was detected in total bilirubin in all the interferent concentrations analyzed with a range of -15 to -21%, although mainly in total bilirubin concentrations higher than 1.5 mg/dL. A significant negative interference was observed in direct bilirubin between 0.3-0.8 mg/dL of -17%, with inconsistent significance in the rest of the range.

CONCLUSIONS

The discoloration of serum samples from patients under treatment with Eltrombopag, due to its metabolites, can interfere positively with creatinine values by more than 20% and negatively with bilirubin values by more than 15%. These interferences must be taken into account for a correct analytical follow-up of these patients.

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W016

A PATIENT WITH COINHERITANCE OF ALPHA-GLOBIN GENE TRIPLICATION AND IVSI-1 (G>A) MUTATION OF BETA-GLOBIN GENE

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BACKGROUND-AIM

Beta thalassemia (β-thal) comprises a heterogeneous group of hemoglobin disorders characterized by a reduction or a complete absence of β-globin gene expression. In areas where β-thal mutations are prevalent and heterogeneous, the spectrum of thalassemia conditions is a continuum from asymptomatic (thalassemia minor), to patients with moderate but transfusion-independent anemia (thalassemia intermedia), to classical transfusion-dependent thalassemia major. Thalassemia intermedia is an infra-diagnosed disease with heterogeneous clinic presentation. It is essential to determine the reasons (or genetic modifiers) for their control and management can be established.

METHODS

CASE REPORT: A 5-year-old Mediterranean infant (Algerian) with asthenia and anorexy referred to the pediatric physician. Laboratory test results were: RBC=4.55x106/µL (4.10 x106-5.20 x106), Hb=8.6 g/dL (11.5-15.5), MCV=60 fL 80-99), MCH=19 pg (27-31). In the peripheral blood smear was seen hypochromic and microcytic RBC's with cells with basophilic stippling, schistocytes and 2 to 100 NRBC per each 100 WBC. Therefore the test result for hemoglobin electrophoresis was the following: HbA0=83%, HbA2=5%, HbF=3.7%.

RESULTS

The molecular studies of β-thal (screening for the most common Mediterranean β-thal mutations using Real-Time PCR) showed a β-thal mutation, IVS-I-1 (G>A), in heterozygous. Compound heterozygous or dominant β-globin mutations were discarded by Sanger DNA sequencing method. This genotype did not justify the unexpected severe phenotype of β-thal carrier suggesting α-globin gene multiplications as genetic modifier. MLPA (multiplex ligation-dependent probe amplification) technique was applied to detect the copy number variations in α-globin genes. The MLPA revealed the αααanti-3.7 triplication in heterozygous.

CONCLUSIONS

CONCLUSIONS: The genotype and phenotype characteristics of the patient described here indicate the need to consider the possibility of a duplicated α-globin allele or even locus in patients with a β-thal heterozygosity who show an unexpectedly severe phenotype. Identification of the α-globin gene multiplications definitely would help physicians in populations with high prevalent thalassemia to provide appropriate genetic counselling.

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W017

MARGINAL ZONE LYMPHOMA OR MANTLE CELL LYMPHOMA

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BACKGROUND-AIM

Marginal zone lymphoma (MZL) and mantle cell lymphoma (MCL), haematology diseases, can appear similar morphologically. For the differential diagnosis, we used lymphocyte typing, karyotype and Fluorescence in situ hybridization (FISH). We present the case of a 69-year-old female patient consulting the emergency room for a fall and disorientation.

METHODS

Complete blood count (CBC) was performed on XN 3000 (Sysmex) according to the supplier's recommendations. We realized bone marrow aspiration and biopsy. Lymphocyte typing was performed on blood by flow cytometry analysis (CMF). Between 0.5 and 1×106 cells were stained for surface antigens with the following antibody combinations labelled with fluorescein isothyocyanate/peridinin chlorophyll-cyanine 5.5/allophycocyanine:
•diagnosis orientation: CD20/CD5/CD19/CD43/CD56/CD3/CD4/CD8/CD10/kappa/lambda;
•Matutes score: FMC7/CD23/CD79b/CD38/CD200 (Matutes score);
•panel CD5: CD103/CD123/CD22/CD24/CD27/CD25/CD11c/CD180/LAIR-1. Antibodies were purchased from Becton–Dickinson (BD), Dako, Beckman Coulter, Sysmex, Clinisciences and Life Technology. CMF was performed on FACSCanto (BD). Bone marrow aspiration and biopsy were sent to CERBA laboratory for karyotype and FISH.

RESULTS

CBC shows a hyperlymphocytosis at 9.6 G/L (normal:1-4). We observe under the microscope a monomorphic population of small to medium sized lymphocytes, mature chromatin, absence of nucleoli, increased N/C ratio, blue cytoplasm with regular contours, cytological aspect without particularities, quite numerous lysed cells. CMF shows 8 G/L of CD19+, CD20+ (high fluorescence intensity), CD5+, monotypic kappa (high fluorescence intensity), CD200+, Cd180+, CD22+ B lymphocytes. This case displayed the translocation t(11;14) and hyper expressed cyclin D1. FISH: 30% of observed cells shows the IgH/CCND1 rearrangement. In view of all the biological arguments and the good general health status of the patient, it was decided to manage a marginal zone type B lymphoproliferative syndrome.

CONCLUSIONS

The t(11;14) translocation with the IgH/CCND1 rearrangement is mainly found in MCL (50-70%) but can also be found in prolymphocytic B-cell leukemia, MZL (10-20%), CLL and myeloma (2-5%). All these diseases involve the B lymphocyte lineage.

Haematology, including haemostasis

W018

DIAGNOSTIC LIMITATIONS OF LEISHMANIASIS IN ARMENIA

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BACKGROUND-AIM

Armenia is considered to be endemic country for leishmaniasis. During the last decade (from 2000) imported and locally-acquired cases of the disease have been registered here. The disease can be found not only in border regions, but also in some regions of the country. The leishmaniases are a group of vector-borne diseases, caused by parasites and are characterized by chronic course, undulant fever, lymphadenopathy, hepatosplenomegaly and pancytopenia. Prognosis of the disease depends on timely diagnosis and appropriate treatment. Diagnosis is mainly based on detection of Leishmania parasite in bone marrow aspirate, also positive IgM by ELISA. Other diseases to consider in the differential diagnosis for leishmaniasis are as follows: acute leukemia, brucellosis, infectious mononucleosis, different types of anemia (iron deficiency anemia, megaloblastic anemia, hemolytic anemia).

METHODS

Complete blood count, bone marrow aspiration (microscopy) and abdominal ultrasound were performed

RESULTS

Anemia was detected in all cases in the study. Neutropenia was detected in 85.7% of patients aged <1 year, 90.9% of patients aged 1-5years, 50% aged 5-15years and 66.6% aged 15-20 years. Thrombocytopenia was detected mostly in age group of 1-5 years (81.8%), in children aged <1 year it was found in 78.5% of cases, in age group 5-15 years 66.7% and in age group 15-20 years 33.3%. Elevated ESR was observed in all patients aged <1year. In age group 1-5 years high ESR rate was detected in 90.7% of cases, 5-15 years 66.6% of cases and in 15-20 years age group 33.3%. Lymphocytosis was detected mostly in <1 year and 1-5 year age groups and was 92.8% and 100% respectively. In age groups 5-15 years and 15-20 years it was found in 66.6% of cases. Splenomegaly was detected more often than hepatomegaly. After bone marrow aspiration the diagnosis of leishmaniasis was confirmed in 21.4% of patients aged <1 year, 18.1% in age group of 1-5 years, 16.6% 5-15 years age group and in 33.4% in age group of 15-20 year. In 64.3% of cases aged <1year only different types of anemia and in 14.3% of cases acute leukemia were detected after bone marrow aspirate examination. In age group of 1-5 years 63.6% and 18.3% of cases were diagnosed with acute leukemia and anemia respectively. In age group of 5-15 years acute leukemia was diagnosed in 33.4% of patients and in 15-20 years age group the percentage was 66.6%.
Results of bone marrow aspiration

CONCLUSIONS

In conclusion, the main signs for initial diagnosis of leishmaniasis are anemia, thrombocytopenia, neutropenia, lymphocytosis, elevated ESR and splenomegaly (Ultrasound). However, bone marrow examination is required for confirmation of diagnosis.

Haematology, including haemostasis

W019

PREVALENCE OF IRON DEFICIENCY ANAEMIA IN OUR HEALTH AREA

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BACKGROUND-AIM

Iron deficiency anaemia (IDA) is a decrease of red blood cells in the blood due to a shortage of iron (Fe), caused by unmet needs (pregnancy, growth spurts, inadequate diet), decreased absorption (atrophic gastritis, celiac disease) and/or increased losses (chronic inflammation, parasitic, gastrointestinal and menstrual infections). The determination of hemoglobin (Hb) alone cannot be used to diagnose Fe deficiency, so other biochemical determinations such as ferritin, serum iron, transferrin, Total Iron Binding Capacity (TIBC) and Iron Saturation Index (ISI) are performed for correct diagnosis. The objective is to identify the prevalence of IDA in our health area.

METHODS

Observational and descriptive study during 2019, which included 51997 hemograms, extracted using our laboratory information system. Admitted patients and urgent requests were excluded and were classified according to age and sex. The cut-off point for Hb was: <12 g/dL females, <13 g/dL males, <12 g/dL 12-15 years, <11.5 g/dL 5-12 years and <11 g/dL in <5 years and for ferritin <15µ/L. Serum iron, ISI, TIBC, transferrin and C-reactive protein results were also reviewed. Hemograms were processed on the Sysmex XN-1000 analyser and the rest of the biochemical determinations on Roche® cobas 8000. Statistical calculations were performed using Medcalc® software.

RESULTS

51997 hemograms from primary care were performed, of which 6705 were anaemic and 2449 were compatible with IDA. The prevalence of IDA is: <13 years 1.1%, 13-60 years 5.7% (1664 were women) and >60 years 3.8%. The proportion of IDA among the anaemias in our area is: <13 years 23.4%, 13-60 years 64.6% and >60 years 16.9%.

CONCLUSIONS

The prevalence of IDA in our health area is low. Likewise, the proportion of IDA among anaemias in our area is 34.9%. It should be noted that between 13-60 years most population were women, coinciding with menstrual loss, growth during adolescence, diet with Fe deficiency and pregnancy. The laboratory plays an important role in the diagnosis of FA through haematological and biochemical tests. Ferritin being an acute phase protein should be assessed with caution, with plasma transferrin and C-reactive protein being useful in patients with chronic inflammation, cancer, liver or kidney disease.

Haematology, including haemostasis

W020

ANALYTICAL VERIFICATION OF THE IMMUNOTURBIDIMETRY METHOD FOR MEASURING C1-INHIBITOR ANTIGEN

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BACKGROUND-AIM

Quantitative or qualitative C1-inhibitor deficiency is the cause of hereditary angioedema, a rare inherited disease manifesting as recurrent and potentially life-threatening swelling episodes. The aim was analytical verification of the automated immunoturbidimetry method for measuring C1-inhibitor antigen and to investigate if results obtained by two different methods and sample types (plasma/serum) are comparable.

METHODS

Analytical verification of the immunoturbidimetry method for quantification of plasma C1-inhibitor antigen was performed on Atellica COAG360 System using N Antiserum to Human C1-Inhibitor (Siemens,Germany). The study included precision testing (repeatability and reproducibility by testing N/T PROT CONTROL PY lot 083345 plain and 1:2 diluted, five days in pentaplicate), estimation of accuracy, reference interval verification (n=20 healthy subjects) and comparison with routinely used radial immunodiffusion method (NOR-Partigen). Forty leftover citrated plasma (BD Vacutainer, USA, with 0.105M Na3citrate, centrifuged at 4000 rpm for 15 minutes) and serum (Vacuette, Greiner bio-one with clot activator, centrifuged at 3500 rpm for 10 minutes) samples were used from patients with requested C1-inhibitor antigen routine laboratory testing.

RESULTS

Results of the accuracy assessment were within the declared range(15%). Coefficients of variation for repeatability (1.3%/1.0%) and reproducibility (1.1%/3.2%) met the manufacturer´s acceptance criteria for normal (≤7.5%) and pathological level (≤10%).All results of the reference interval verification were within the manufacturer´s stated interval (0.170-0.290 g/L).Method comparison (measurement range: 0.072-0.384g/L) using Passing-Bablok regression analysis yielded a regression equation y=-0.03(95% CI -0.10 to 0.01)+1.03(95% CI 0.85 to 1.30)x,(correlation coefficient: 0.813,P<0.001),indicating no constant nor proportional difference in C1-inhibitor antigen results.

CONCLUSIONS

Analytical verification of the immunoturbidimetric method showed excellent performance, demonstrating that plasma C1-inhibitor antigen results were comparable to serum values determined by radial immunodiffusion.The Atellica COAG360 System offers the unique possibility to analyse both C1-inhibitor activity and antigen in plasma samples on the same platform.

Haematology, including haemostasis

W021

MONOCYTE DISTRIBUTION WIDTH (MDW) VALUES MEASURED IN K2-EDTA AND K3-EDTA TEST TUBES

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BACKGROUND-AIM

Monocyte Distribution Width (MDW) is a novel hematology parameter, which represents a measure of change in the monocyte size distribution. Morphological changes of monocytes in sepsis show greater heterogeneity of the monocyte population and thus a higher MDW value. MDW provides significant added value along with currently used sepsis markers (WBC, PCT, CRP, IL-6) for early sepsis screening. The clinical MDW cut-off of 20.0 was established through a blinded prospective multi-center pilot study using K2-EDTA venous whole blood samples. According to this study, MDW values >20.0 should raise suspicion that sepsis is present or will develop in patients within twelve hours of the Emergency Department (ED) presentation. The type of EDTA salt may affect the accuracy of cell counting and sizing. The aim of our study was to evaluate the impact of Beckton-Dickinson (BD) blood collection tubes containing K2-EDTA and K3-EDTA as anticoagulants on MDW values.

METHODS

Blood samples from 158 apparently healthy individuals undergoing annual health checkups were collected in K2-EDTA and simultaneously in K3-EDTA test tubes. All samples were analyzed for CBC with differential within 30 min after collection on a Beckman Coulter Unicel DxH 900 hematology analyzer. Data were tested for normality using the Kolmogorov–Smirnov test. The significance of differences between samples was assessed by Wilcoxon's paired test. $P < 0.05$ was considered statistically significant. Data are presented as medians and interquartile ranges. Bland-Altman plotting was performed to assess the comparability of results. Statistical analysis was performed using MedCalc 14.8.1.0 statistical software.

RESULTS

The study included 158 participants, age 30 (18–58), 91 (57,6%) males. For K2-EDTA tubes median MDW value was 16,25 (15,38–17,44) and for K3-EDTA tubes 17,11 (16,18–18,42). A statistically significant difference was found comparing MDW values in K2-EDTA and K3-EDTA ($P<0.001$). Bland-Altman analysis revealed statistically significant constant (mean -0,8; 95%CI -1,03 to -0,59) and proportional differences (mean -4,8%; 95%CI -6,10 to -3,52). Limits of agreement (±1.96 SD of differences) ranged from -3.6 to 1.9 for constant difference and -20,9 to 11.2% for proportional difference, respectively. Most of the values were lower when measured in K2-EDTA then in K3-EDTA.

CONCLUSIONS

K2-EDTA is recommended as the anticoagulant of choice for routine hematology testing by the International Council for Standardization in Hematology (ICSH) and Clinical Laboratory Standard Institute (CLSI). Although K2-EDTA is the anticoagulant of choice, K3-EDTA tubes are still in widespread use. Our study provides evidence that there is a statistically significant difference between K2-EDTA and K3-EDTA MDW measurements.To accurately use of MDW as additional marker for early sepsis screening, it is necessary to perform clinical outcome studies based on the impact of K3-EDTA on MDW measurements.

Haematology, including haemostasis

W022

ANALYSIS OF THE DIFFERENCES BETWEEN TWO METHODS FOR ERYTHROPOYETIN

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BACKGROUND-AIM

Erythropoietin (EPO) is a glycoprotein that acts as the main stimulator of erythrocyte formation. In humans, it is synthesized for the most part in mesangial cells and in interstitial cells of the kidney. The quantification of EPO is useful in the diagnosis and follow-up of Anemias and Polycythemias. The main objective is the assessment of the interchangeability of two methods for the quantification of EPO.

METHODS

A retrospective analysis of 44 samples of patients of both sexes was carried out, with 54.54% being men and 45.46% women (32 – 72 years). Range = 2.88 - 615.16 mU / mL. The quantification of EPO by chemiluminescence in the UniCel™ DxI 800 Immunnoassay system, Beckamn Coulter® was used as a reference method and it was compared against the chemiluminescence method in the Inmulite 2000 Xpi, Siemens Healthcare®. To obtain results, the SPSS® v23.0 computer program was used a regression analysis, determination of the correlation coefficient and a Bland-Altman analysis to assess the differences between both methods. In addition, taking the reference values provided by the manufacturers, the patients were classified dichotomously by the EPO result if they were within this range or not.

RESULTS

Both techniques showed a good correlation (r=0.988). The resulting equation, y = 0.9209x (CI95% = 0.88 – 0.96) - 0.023 (CI95% = -5.89 – 5.83). Bland-Altman analysis shows a mean of difference = -4.42 ± 19.83.

CONCLUSIONS

Based on the results obtained after the statistical analysis, there are no differences in the determination of EPO by the different tests. The evaluated methods do not present constant type differences but they do present proportional differences since the 95% confidence interval for the ordinate at the origin does not include the value 1. This proportional difference increases as the concentration of EPO increases. . The implementation of the new method requires prior assessment of the reference values provided. The capacity to classify patients is 86.36% in this series, with 6 in which the classification is not correlated, especially in patients with values below the lower limit provided.

Haematology, including haemostasis

W023

LARGE PLATELET FRACTION (LPF) - A NOVEL PARAMETER MEASURED BY YUMIZEN H2500 AND ITS CORRELATION WITH IMMATURE PLATELET FRACTION (IPF) BY SYSMEX XN1000

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BACKGROUND-AIM

Platelet analysis and estimation is simple yet a complex process for various pathological conditions. Platelet activity can correlate with the platelet size and the platelet numbers. The platelet size can be assessed by various platelet indices and technological derivatives. Impedance technology is widely used however this has a limitation specially in thrombocytopenia cases with large or giant platelets, red cell fragmentation, when a platelet histogram cannot be drawn properly to derive an accurate platelet count or its indices. these conditions form the major chunk of platelet associated dilemmas in the laboratory set up. The indices could often help to distinguish hyper-destructive thrombocytopenia and hypo-productive thrombocytopenia very easily. The aim of the study was to evaluate the analytical efficiency of a novel methodological solutions, used in haematology analyser Yumizen H2500, to measure the large platelet fraction (LPF) and to compare with a immature platelet fractions (IPF) measured by Sysmex XN 1000i.

METHODS

Total 328 patients venous blood samples were selected randomly using different criteria like MPV >11.5 fL, MPV <11.5 fL, schistocytes flag or MCV <60 fL. All the samples were processed in duplicate within 4 hours of phlebotomy using both haematology analysers in parallel for platelet determination. The analyser XN1000i uses laser flow cytometer based on florescent dye. The analyser Yumizen H2500 uses a combination of optical extinction and impedance technology. A number of parameters were available from both the analysers. After outlier exclusion, 272 samples were statistically analysed and LPF (available in Yumizen H2500) and IPF (available in XN 1000i) were correlated.

RESULTS

A good correlation was observed between the novel LPF by Yumizen H2500 & IPF by Sysmex XN 1000i with a correlation coefficient (r2) 0.933. The regression equation (y = 0.89x + 0) a good agreement between both parameter with intercept equal to 0 (95% CI -0.192 to 0.192) and slope equal to 0.89 (95% CI 0.846 to 0.933) and an acceptable bias of -11.03% (95% CI -7.4 to -14.8).

CONCLUSIONS

Large platelet counting could be an important challenge in specific haematology disorders. The novel parameter LPF can help laboratories in the critical decision making and is comparable to IPF. LPF can be a more appropriate nomenclature as both LPF and IPF gives the same information but using different methodology.

Haematology, including haemostasis

W024

G6PD AND GAPDH AS POTENTIAL DIAGNOSTIC BIOMARKERS FOR IRON DEFICIENCY ANEMIA

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BACKGROUND-AIM

Iron has vital metabolic functions such as oxygen transport, electron transfer, cofactor, and is a structural component of heme-containing proteins. Therefore, metabolic damage occurs in its deficiency. Iron deficiency anemia (IDA) is worldwide common anemia based on dietary iron deficiency and/or iron absorption problems. Ferritin is used as a biomarker in the diagnosis of IDA. In this study, we aimed to observe the usability of glucose 6 phosphate dehydrogenase (G6PD) and glyceraldehyde 3 phosphate dehydrogenase (GAPDH) enzyme activities as biomarkers instead of ferritin in IDA patients. We examined the affair between G6PD and GAPDH enzyme activities and other blood and oxidative stress parameters in patients with IDA.

METHODS

The study groups consisted of 2 groups aged between 18-65 years, namely the healthy control group (n=100) and the IDA patients group (n=80). Analyzes were performed on blood, hemolysate, and serum using spectrophotometric methods. Statistical analyzes were performed in R (v.4.1.0).

RESULTS

According to the results we obtained, the G6PD enzyme activity in the patient and control groups was found to be 18.52 ± 3.1 IU/gHb and 14.04 ± 1.6 IU/gHb, respectively ($p < .001$). In addition, a significant difference was found in the comparison of GAPDH, glutamate dehydrogenase, malate dehydrogenase, catalase, thiol-disulfide, and TOS levels between the patient and control groups. The area under the ROC curve using G6PD, GAPDH, and ferritin differentiating patients and controls was 0.914 (%95 CI = 0.87-0.96), 0.928 (%95 CI = 0.88-0.97), and 0.88 (%95 CI= 0.83-0.93) respectively.

CONCLUSIONS

According to these results, we consider that G6PD and GAPDH enzyme activities can be used as differential diagnostic criteria between IDA and other anemia types instead of ferritin. Nevertheless, further study with a larger sample size is necessary to determine this hypothesis.

Haematology, including haemostasis

W025

THE MONOCYTE TO LYMPHOCYTE RATIO IN END STAGE CHRONIC KIDNEY DISEASE

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BACKGROUND-AIM

The Monocyte to lymphocyte ratio (MLR) is represented as relatively new marker of the systemic inflammatory responce and as such could be associated with cardiovascular disease morbidity and mortality in chronic kidney disease (CKD) patients. The aim of the study was to evaluate values of MLR index in end stage CKD patients on hemodialysis programme.

METHODS

The study was carried out at the Center of Laboratory Medicine, Clinical Center of Vojvodina and included 35 hemodialysis patients who havent had acute inflammatory process and 30 healthy subjects, age and gender matched to the examined group. Complete blood count results and c-reactive protein (CRP) was determined to all patients. MLR was calculated as monocytes count devided to lymphocytes count.

RESULTS

Examined group had significantly higher CRP (5.27±0.23 mg/L vs. 2.14±0.73 mg/L; p=0,009). Hemodialyse group had higher values of monocyte absolute count and lower lymphocyte absolute count, but without statistical significance (p=0.71 and p=0.17 respectively). However, in the hemodialysis group MLR values were significantly higher (0.60±0.09 vs 0.19±0.05; p=0.02).

CONCLUSIONS

Hemodialysis patients had significantly higher values of MLR.

Haematology, including haemostasis

W026

ENUMERATION OF CD34+ HEMATOPOIETIC STEM CELLS USING FLOW CYTOMETRY

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BACKGROUND-AIM

Stem cells for autologous and allogenic transplantation could be obtained from several sources including: bone marrow, peripheral blood or cord blood. Accurate flow cytometric enumeration of CD34+ hematopoietic stem cells is routinely used in clinical settings, especially to monitor progenitor cell mobilization and apheresis.

METHODS

In Clinical Center of Vojvodina,enumeration of CD34+ cells was established from April 2021 as a routine laboratory analysis.This method is performed on BD FACSCanto II flow cytometer using CD45 V450 /CD34 PE protocol. From 19th of April, 2021 eight patients were analysed in order to monitor progenitor cell mobilization and apheresis process (seven for autologuous and only one for allogenic transplantation procedure). All patients were analysed in duplicates. After the flow cytometry analysis result is presented as the absolute number of cells in a microliter of apheresis product. The final result is obtained by applying the following formula: apheresis product volume (expressed in liters) multiplied with absolute number of cells in a microliter of apheresis product and devided with body weight (expressed in kilograms). Recomended reference range for stem cell counts is 2-5 x 106 cells / kg of body weight.

RESULTS

For seven patients who underwent apheresis and collection procedure for autologuous transplatation the mean value of obtained stem cells in apheresis product were: 13.5 x 106 cells /kg of body weight. Compared to recommended values, two of seven patients had lower stem cell counts values. Patient who was candidate for allogenic transplantation had 10.0 x106 cells /kg of body weight.

CONCLUSIONS

Enumeration of viable CD34+ using this flow cytometry protocol can be used in daily routine and as such could represents a prerequisite for successful transplantation procedure.

Haematology, including haemostasis

W027

ADDITIONAL HAEMATOLOGICAL PARAMETERS AS A PREDICTOR OF BACTEREMIA IN PATIENTS AFTER HEART TRANSPLANTATION

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BACKGROUND-AIM

Infectious complications are one of the leading causes of mortality after the heart transplantation. However, the diagnosis of bacteremia remains challenging due to immunosuppressive therapy. This study was aimed to evaluate the utility of additional haematological parameters in diagnosis of bacteremia.

METHODS

Blood samples from heart transplantation recipients with bacteremia (n=11), without bacteremia (n=73) and healthy control subjects (n=29) were obtained for cell count on the same day of culture collection. In addition to the routine count, VCS (V-volume, C-conductivity, S-scatter) parameters were determined using automated haematology analyzer Unicel DxH800 Coulter (Beckman Coulter). Statistical analysis was performed using StatSoft STATISTICA 10.0 for Windows.

RESULTS

In neutrophils (NE) mean (MN) median angle light scatter (MN-MALS-NE), lower median angle light scatter (MN-LMALS-NE) were lower in the bacteremia group versus non-bacteremia and control groups ($p<0.05$). Axial light loss (MN-AL2-NE) was higher in the bacteremia group ($p<0.05$). Standard deviation (SD) of NE volume (SD-V-NE) was higher in the bacteremia group (23.2±5.6) versus the non-bacteremia (19.3±3) and control (16.5±1.1) groups ($p=0.01$ and $p<0.01$ respectively). SD of axial light loss (SD-AL2-NE) was higher in the bacteremia group (17.1±5.4) versus the non-bacteremia (12.9±2.4) and control (9.7±0.9) groups ($p=0.04$ and $p<0.01$ respectively). In monocytes (MO) MN volume (MN-V-MO) was higher in the bacteremia group (184.4±8.07) versus the non-bacteremia (177.7±6.9) and control (167.0±5) groups ($p<0.001$). SD of MN MO volume (SD-V-MO) was higher in the bacteremia group (27.5±5.3) versus the non-bacteremia (22.1±4.9) and control (18.4±1.8) groups ($p<0.01$). SD of MO axial light loss (SD-AL2-MO) were higher in bacteremia group (26.2±8.8) versus the non-bacteremia (18.6±7.8) and control (11.4±1) groups ($p<0.01$).

CONCLUSIONS

In our study we confirmed the benefits of using VCS parameters to predict bacteremia. Standard deviation of mean monocyte volume was recognized as the most promising marker. As it is easily to determine this marker on a routine basis it can be incorporated into decision-making rules for early detection of infectious complications.

Haematology, including haemostasis

W028

THE LMWH-CALIBRATED STA LIQUID ANTI-XA-ASSAY: UTILITY TO EXCLUDE RESIDUAL DOAC-ACTIVITIES.

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BACKGROUND-AIM

The use of direct oral anticoagulants (DOACs) is growing rapidly. Besides critical clinical situations, which occur increasingly frequently and require an urgent laboratory assessment of Anti-Factor Xa (Anti-Xa)-activity, current German guidelines require the exclusion of DOAC-activity prior to hip joint close femur fracture treatment procedures. A corresponding resolution of the Federal Joint Committee (GBA) came into effect on April 8, 2021 as an addition to the regulations on the GBA-SOP "Handling of anticoagulant medication". The resolution defines an adequate laboratory test as possible alternative if anamnestic data cannot be collected reliably. Taken together there is the need for a rather universal test to exclude residual Anti-Xa-activity instead of drug-specific calibrated Anti-Xa-assays, not available area-wide.

METHODS

During evaluation of the low molecular weight heparin (LMWH)-Anti-Xa-assay (STA LIQUID ANTI-Xa-Assay) on a STA Max 3-System (both STAGO Deutschland GmbH, Düsseldorf, Germany) we applied DOAC-specific calibrated tests as well as the LMWH-calibrated assay on a total of 163 samples (Apixaban n=65, Rivaroxaban n=51, Edoxaban n=47).

RESULTS

All DOAC-samples disclosing <30ng/mL with drug-specific calibration revealed a LMWH-activity ≤0.35IU/ml. For DOAC-specific concentrations <100ng/ml results show a linear correlation: Apixaban (n=36) r^2=0,983 | Rivaroxaban (n=27) r^2=0,955 | Edoxaban (n=37) r^2=0,985. The correlation loses linearity at higher DOAC-concentrations. At concentrations close to or above intoxication levels, the LMWH-calibrated Anti-Xa-Assay exceeds its upper limit of quantification (ULOQ) preventing a proper statistical assessment.

CONCLUSIONS

In accordance with current published results for analog tests, our data indicate that the LMWH-calibrated STA LIQUID ANTI-Xa-Assay is an acceptable universal Anti-Xa-activity-assay to exclude clinically relevant DOAC-activities. Applying a cut-off of 0.35IU/ml the assay enables to discriminate concentrations <30ng/mL for all three oral Anti-Factor Xa-compounds currently available.
At DOAC-concentration >400ng/mL (n=12) the LMWH-calibrated STA LIQUID ANTI-Xa-Assay exceeds its ULOQ. This read-out might be helpful in situations, where an intoxication needs to be clarified.

Haematology, including haemostasis

W029

2ND GENERATION OF SOLUBLE TRANSFERRIN RECEPTOR ASSAY – CONSEQUENCES FOR THE INTERPRETATION OF THE 'THOMAS' PLOT

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BACKGROUND-AIM

The 'Thomas' plot is a helpful diagnostic tool for clinicians to evaluate and monitor the iron status of patients. For evaluation, CRP, serum ferritin, the hemoglobin of reticulocytes (Ret-He) and the soluble transferrin receptor (sTfR) are measured and the calculated ferritin index (sTfR/lg Ferritin) is plotted against Ret-He. Cut-offs for the ferritin index were initially defined dependent on the inflammatory status (evaluated by CRP). Furthermore, the cut-offs depend on the method used for measurement of sTfR. In 2021, Roche Diagnostics launched a new assay for sTfR measurement. Here we evaluated the comparability of the latest generation of the sTfR assay with the first generation and the consequences for cut-offs used in the Thomas plot.

METHODS

Measurment of sTfR, ferritin, CRP was done using a Cobas8000 system. Ret-He was determined using a Sysmex XN9000 system.

RESULTS

Initially, 69 samples from the clinical routine diagnostics were measured with both sTfR assays (range of the analyzed samples 0.60-11.44 mg/l). We detected an excellent correlation between the two assays (linear regression, R^2=0,974), however, the values measured with the 2nd generation sTfR assay were consistently lower compared to the 1st generation ($p<0.001$). We also calculated the correlation for ferritin index. As expected, the correlation was also very good (linear regression; R^2=0.989), but the ratio calculated with the sTfR-values of the new assay were around 15% lower compared to the ratios calculated with sTfR-values of the old assay ($p<0.001$). To estimate how many samples might be affected by this discrepancy between the old and the new assay, we retrospectively analyzed a dataset of all Thomas plots between 2016-2021 in our lab. Depending on the CRP value, different cut-offs for the ferritin index should be applied (CRP<5 mg/l: 3.2; CRP>5 mg/l: 2.0). Based on the results of direct comparison of the two sTfR assays we defined a range to identify possibly critical affected samples. In total we analyzed 15592 datasets. 813 (5.21%) of these samples were in the range to be possibly falsely assigned to the wrong quadrant by using the new generation sTfR-assay without changing the cut-offs. In our dataset, around 80% of these critical samples had Ret-He values > 1.74 fmol, which would lead to a false assignment from quadrant 2 to quadrant 1 and therefore a latent iron deficiency could be missed. According to the direct comparison of the two assays we changed our cut-offs for the Thomas plot in the following way: for CRP<5 mg/l from 3.2 to 2.8 and for CRP>5 mg/l from 2.0 to 1.8.

CONCLUSIONS

Taken together the present data show that the new generation sTfR-assay from Roche Diagnostics leads to lower sTfR values. This is of potential relevance for ~5% of the samples in our cohort of patients from a university hospital and therefore the cut-off values for the ferritin index need to be reevaluated for correctly diagnosing the iron status of the patients.

Haematology, including haemostasis

W030

THROMBOELASTOMETRY AS GUIDANCE FOR BLOOD MANAGEMENT IN PATIENTS UNDERGOING CARDIAC SURGERY

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BACKGROUND-AIM

Viscoelastic tests (rotational thromboelastometry, ROTEM), together with the implementation of a specific algorithm for coagulation management in cardiac surgery, enable perioperative coagulopathy to be better controlled.

METHODS

Retrospective cohort study including 675 patients who underwent cardiac surgery with cardiopulmonary bypass.
The incidence of allogeneic blood transfusions and clinical postoperative complications were analyzed before and after ROTEM implementation.

RESULTS

Following viscoelastic testing and the implementation of a specific algorithm for coagulation management, the incidence of any allogeneic blood transfusion decreased (41.4% vs 31.9%, p=0.026) during the perioperative period. In the group monitored with ROTEM, decreased incidence of transfusion was observed for packed red blood cells (31.3% vs 19.8%, p=0.002), fresh frozen plasma (9.8% vs 3.8%, p=0.008), prothrombin complex concentrate administration (0.9% vs 0.3%, p=0.599) and activated recombinant factor VII (0.3% vs 0.0%, p¼0.603). Increased incidence was observed for platelet transfusion (4.8% vs 6.8%, p=0.813) and fibrinogen concentrate (0.9% vs 3.5%, p=0.066), tranexamic acid (0.0% vs 0.6%, p=0.370) and protamine administration (0.6% vs 0.9%, p=0.908). Similar results were observed in the postoperative period, but with a decreased incidence of platelet transfusion (4.8% vs 3.8%, p=0.813). In addition, statistically significant reductions were detected in the incidence of postoperative bleeding (9.5% vs 5.3%, p=0.037), surgical reexploration (6.0% vs 2.9%, p=0.035), and length of Intensive Care Unit (ICU) stay (6.0 days vs 5.3 days, p=0.026).

CONCLUSIONS

The monitoring of hemostasis by ROTEM in cardiac surgery, was associated with decreased incidence of allogeneic blood transfusion, clinical hematologic postoperative complications and lengths of ICU stay.

Haematology, including haemostasis

W031

HEVYLITE: AN ALTERNATIVE ASSAY TO MONITOR MONOCLONAL INTACT IMMUNOGLOBULINS IN PATIENTS WITH MULTIPLE MYELOMA.

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BACKGROUND-AIM

The detection of the monoclonal protein (MP) in serum by electrophoresis (SPE) and immunofixation (IFE) is used to assess treatment response in multiple myeloma (MM). Hevylite® is an automatized assay that measures specific pairs of heavy+light chains of immunoglobulins, providing a precise quantification of the involved HLC pair (iHLC), which is the MP, and the uninvolved HLC pair of the same isotype (i.e., IgGκ in a IgGλ patient). This allows a sensitive detection of clonality through the iHLC/uHLC ratio. The uHLC pair quantification also allows the detection of a new isotype-matched immunosuppression (IMI). AIM: To assess the performance of HLC in relation to SPE/IFE to monitor intact immunoglobulin MM (IIMM).

METHODS

Hevylite® was performed using a SPAplus from 2012 to 2017 and an Optilite system since then. Normal reference ranges indicated by the manufacturer were used. SPE was performed by the Capillarys Hydrasys Focusing (Sebia).

RESULTS

Hevylite® was reviewed in 40 IIMM patients. All 30 (100%) newly diagnosed IIMM patients had an abnormal HLC ratio at baseline. 27/30 (90%) presented decreased levels of the uHLC pair (IMI) (i.e., IgGκ in a IgGλ patient), 24 (80%) of which with uHLC <50% of the lower limit of the reference range (severe IMI). Good correlations were obtained between the MP concentration measured by SPE and dHLC=iHLC-uHLC (spearman coefficient=0.9125; n=987), and between total immunoglobulin concentration and the sum of iHLC+uHLC (spearman coefficient=0.92; n=1095). PFS was defined as time from the end of the treatment/post-ASCT for transplanted patients until progression or death from any cause. 29 1st, 10 2nd, and 6 3rd lines of therapy from 36 patients were included in survival analysis. Patients with ≥VGPR had significantly higher PFS than patients with <VGPR by both criteria (IMWG: p=0.0091; HLC: p<0.0002; Logrank test). Median PFS for patients in <VGPR as defined by HLC was shorter than those defined by the IMWG criteria (135 vs. 303 days), suggesting that HLC identified patients with higher risk of progression.

CONCLUSIONS

Hevylite® precisely quantified the MP, allowing a correct response assessment as demonstrated by its ability to discriminate a group of patients (≥VGPR) with longer PFS.

Haematology, including haemostasis

W032

PROGNOSIS VALUE OF REACTIVE LYMPHOCYTES CIRCULATING IN PATIENTS WITH COVID-19 INFECTION

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BACKGROUND-AIM

Reactive lymphocytes (RL) circulating in peripheral blood (PB) have been reported in patients with COVID-19 infection. This study aims to analyse if patients in which these RL are detected in PB show differences related to the prognosis.

METHODS

Clinical and laboratory findings from a total of 185 patients infected with COVID-19 were compared. 106 patients showed RL in PB (RL+) and these cells were absent in the remaining 79 (RL-). Blood samples were collected on admission, being several haematological and biochemical parameters measured. Blood counts and biochemical parameters were analysed in Advia®2120i and Atellica®, respectively. Digital images were acquired in CellaVision®DM96 using PB smears stained with MGG. Mann Witney U test and Fisher test were used.

RESULTS

Median values (years) were 59±17 in patients RL+, and 71±14 in RL-, p<0.001. RL showed a large-medium size, regular or kidney-shaped nucleus with a spongy chromatin pattern and a deep blue cytoplasm with occasional small vacuoles and reached mean values of 0.18x10e9/L.
Dyspnoea was more frequent in the RL- group (p=0.07). Haemoglobin, red blood cell and lymphocyte (L) counts were higher in RL+ (p<0.001). In the RL- patients we found increased values in neutrophils (N), N/L ratio, D-dimer, cardiac troponin I, procalcitonin, glomerular filtration rate, blood urea nitrogen, direct bilirubin, alkaline phosphatase, direct bilirubin and LDH (p<0.001). Other parameters increased were ratio platelets/leukocytes (p<0,006), monocytes (p=0,002), creatinine (p=0.005) and GGT (p=0.014). In addition, RL- patients showed significant decreased values in total protein and albumin (p<0.001).
A high number of RL- patients received antibiotics (p<0.001), antifungals (p=0.013) and immunosuppressants (p=0.002). Number of hospitalization days and period between the onset of symptoms and discharge was longer for RL- patients (p<0.001). In this group, patients that required admission to the ICU or requiring mechanical ventilation and mortality was higher (<0.001).

CONCLUSIONS

We found that RL detection in PB smear is related to a better prognosis of the COVID-19 infection suggesting an abundant production of virus-specific T cells, thus explaining the better outcome of patients showing these cells in blood.

Haematology, including haemostasis

W033

EFFICIENT MORPHOLOGICAL FLAGGING BY THE ABBOTT ALINITY HQ

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BACKGROUND-AIM

The Abbott Alinity hq high throughput hematology analyzer provides a 6-part WBC differential, including immature granulocyte and nucleated red blood cell concentration with every CBC. Morphological flags are generated to detect the presence of blasts, variant lymphocytes, neutrophilic left shift, RBC fragments and PLT clumps and to alert the operator for the need of blood film review. The aim of this study was to assess the morphological flagging performance of the Alinity hq.

METHODS

Patient samples (n=397) with normal and abnormal CBC results were tested at six clinical sites and one Abbott internal site. Three stained blood films were prepared for each sample and scanned by CellaVision DM9600. Two qualified technologists used the DM9600 to independently perform a 200-cell WBC differential count and reviewed the RBC and PLT morphology (with the exception of PLT clumps) for each sample, using different blood films. If the review of the two technologists did not agree, a third independent reviewer performed an adjudication using the third stained blood smear. PLT clumps were assessed by independent review of blood films by two qualified technologists using manual microscopy. The performance of Alinity hq BLAST, VAR LYM, Left Shift, RBC Frag and PLT Clump flags was assessed based on the microscopic WBC differential and PLT and RBC morphological results. Results were categorized as True Positive, False Positive, False Negative, and True Negative, and percent sensitivity, specificity and efficiency were calculated based on binomial distributions.

RESULTS

The cohort included 8 samples with > 1% blasts, 24 samples with > 5% variant lymphocytes, 41 samples with >6% band neutrophils and 2 and 4 samples with PLT clumps and schistocytes, respectively. Sensitivity, specificity and efficiency of the BLAST flag was 100%, 92.80% and 92.95%, respectively. The pooled sensitivity, specificity and efficiency of all flags was 72.60%, 80.56% and 79.09%, respectively, with a 45.69% positive predictive value and 92.88% negative predictive value.

CONCLUSIONS

The Alinity hq morphological flagging is in line with published performance characteristics of modern hematology analyzers, meeting the needs of today's clinical laboratories.

Haematology, including haemostasis

W034

ANALYTICAL PERFORMANCE CHARACTERIZATION OF THE ABBOTT ALINITY HQ RESEARCH PARAMETERS

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BACKGROUND-AIM

The Abbott Alinity hq hematology analyzer reports several research parameters, including some unique measurands made possible by the advanced Multi Angle Polarized Scatter Separation (MAPSS™) technology. Many of these measurands have been suggested to have potential clinical utility; however, performance characteristics for these measurands are not available. The aim of this evaluation was the analytical performance characterization of the Abbott Alinity hq research parameters.

METHODS

Short term imprecision was assessed on 20 patient specimens across five analyzers. Long term imprecision was evaluated using Alinity h-series 29P Controls in a 20-day study, and reproducibility was assessed using three analyzers according to CLSI EP05-A3. Linearity of selected measurands was assessed per CLSI EP06-A2, and interference by bilirubin, triglycerides, cholesterol and free hemoglobin was evaluated.

RESULTS

WBC viability fraction (WVF), RDW-SD, % microcytic, macrocytic, hypochromic and hyperchromic RBC (%MIC, %MAC, %HPO, %HPR), hemoglobin distribution width (HDW), cellular hemoglobin concentration mean (CHCM), calculated hemoglobin (cHGB), mean corpuscular volume of reticulocytes (MCVr), mean cellular hemoglobin concentration of reticulocytes (CHCr), plateletcrit (PCT), platelet distribution width (PDW) and absolute concentration of reticulated platelets (rP) were assessed.
Short term imprecision was < 3% for WVF, RDW-SD, cHGB, HDW, CHCM, PDW, PCT, MCVr and CHCr. The %CV ranged between 6.1 and 16.9% for rP, %MIC, %MAC, %HPO and %HPR. Long term imprecision gave similar results. cHGB and PCT showed linearity with a slope of 0.94-0.98 for cHGB and 1.02-1.04 for PCT on three analyzers. rP demonstrated lower slope of 0.93-0.97 and correlation coefficient of 0.73-0.80. No significant interference was detected by conjugated and unconjugated bilirubin up to 80 mg/dL, triglycerides and cholesterol up to 400 mg/dL, and hemoglobin up to 500 mg/dL. Free hemoglobin did not interfere with cHGB measurements.

CONCLUSIONS

Alinity hq research parameters demonstrated high level of repeatability, stable, reproducible results over time and across analyzers, linear performance in the assessed ranges, and lack of interference by common interfering factors.

Haematology, including haemostasis

W035

ADULT AND PEDIATRIC REFERENCE RANGES FOR ALINITY HQ RESEARCH PARAMETERS

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BACKGROUND-AIM

The Abbott Alinity hq hematology system utilizes advanced MAPSS™ technology and fluorescence flow cytometry. It reports the following research use only (RUO) parameters: WBC viability fraction (WVF), RDW-SD, percent microcytic, macrocytic, hypochromic and hyperchromic RBC (%MIC, %MAC, %HPO, %HPR), hemoglobin distribution width (HDW), cellular hemoglobin concentration mean (CHCM), calculated hemoglobin (cHGB), mean corpuscular volume of reticulocytes (MCVr), mean cellular hemoglobin concentration of reticulocytes (CHCr), plateletcrit (PCT), platelet distribution width (PDW), and absolute concentration of reticulated platelets (rP). Several of these RUO parameters may have potential clinical applications based on published literature. The aim of this study was to assess the reference ranges for these measurands.

METHODS

For the establishment of adult reference ranges, 286 samples were used from apparently healthy subjects (22-88 years). The distribution of the data was assessed separately in males and females by the Shapiro–Wilk test and the central 95th percentile ranges were calculated per CLSI EP28-A3c guideline using a non-parametric method. Difference between males and females was assessed by the Mann-Whitney test. The pediatric population (n=176) was divided into neonates (birth to <1 month), infants (1 month to <2 years), children (2 to <12 years) and adolescents (12 to 21 years).

RESULTS

The distribution of the data was non-normal for most parameters, except for HDW, CHCM, MCVr, and CHCr in the adult male population, and HDW, CHCM, cHGB, and PDW in the adult female population. Significant differences were observed between males and females for %HPR, HDW, CHCM, cHGB, CHCr, and PCT, ($p<0.05$), and no differences were observed for WVF, RDW-SD, %MIC, %MAC, %HPO, MCVr, PDW, and rP.
For pediatric populations, the minimum, maximum, mean, and median of the results were calculated for each measurand in each age group separately: neonates: n=36, infants: n=44, children: n=43, adolescents: n=53.

CONCLUSIONS

Reference ranges for the Alinity hq research parameters were established in this study. These ranges can serve as a guide for Alinity hq users and as a basis for comparison with other analyzers and may facilitate scientific research using these parameters.

Haematology, including haemostasis

W036

POINT-OF-CARE DOAC DIPSTICK TEST FOR SCREENING OF DOAC IN URINE

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BACKGROUND-AIM

Monitoring of DOAC concentration in blood is useful in certain emergency situations (before thrombolysis, surgery and other invasive procedures). Implementation of DOAC blood concentration assays to clinical practice is limited due to methods` complexity, cost etc. Alternatively, patients can be screened for DOAC usage from 1-2 hours after oral intake by urine analysis.
The aim of this study was to evaluate the new point-of-care DOAC Dipstick test for qualitative assessment of presence of DOACs in urine.

METHODS

The study was performed at the North Estonia Medical Centre and was approved by the Tallinn Medical Research Ethics Committee. Samples from 23 patients receiving apixaban (APBN), rivaroxaban (RXN) or dabigatran (DBTN) were obtained and analyzed in the lab. The control group was compiled of 10 volunteers without DOAC intake history. DOAC concentrations in plasma and urine were determined by chromogenic assays (STA-ECA II for DBTN and STA-Liquid Anti-Xa for RXN and APBN; all with dedicated calibrators) on automated coagulation analyzer STA-R Evolution (Diagnostica Stago, France). The safe-for-treatment cut-off for DOACs of 30 ng/mL in plasma is approved in our hospital. DOAC Dipstick (DOASENSE GmbH, Germany) qualitative assay to evaluate the presence or absence of RXN, APBN or DBTN in urine was carried out by visual investigation, comparing results with the reference scale by three independent laboratory technicians and DOASENSE Reader. Positive and negative controls (DOASENSE Control Urines) were also analyzed.

RESULTS

Total 23 patients (males 10, females 13) were included. Average age was 71.4 years (range 38-88). A median DOAC concentration in plasma was for DBTN 48.2 (range 46–52) ng/mL, for RXN 159.5 (range 3-456) ng/mL and for APBN 126.0 (range75–275) ng/mL.
The functionality and quality of the DOAC Dipstick were proved by two levels of DOASENSE Control Urines.
Presence or absence of DOAC, assessed by DOASENSE Reader, was in agreement with DOACs quantitative measurements in urine in most cases. In 2 cases test was positive for both types of DOACs, which is unlikely that patients took two types of DOACs simultaneously.
Visual investigation of the DOAC Dipstick has demonstrated somewhat inter-individual variability for factor Xa-inhibitor pad: urine colors were interpreted falsely in 2 cases. Interpretation of creatinine pad color had disagreement between investigators in 5 cases.
Concentrations of DOAC below the 30 ng/mL in plasma were found in 8.7% (2/23) samples, but urine test was positive. The urine incubation time in bladder was not correctly documented in all cases.
Control group results were visually correctly interpreted and were in agreement with DOASENSE Reader results.

CONCLUSIONS

DOAC Dipstick is useful for rapid assessment of absence of DOACs in urine. Nevertheless, to aid clinical decisions positive results should be confirmed with quantitative assay for DOACs in the blood.

Haematology, including haemostasis

W037

SECONDARY HEMOPHAGOCYTIC SYNDROME: THE IMPORTANCE OF EARLY DIAGNOSIS

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BACKGROUND-AIM

Hemophagocytic syndrome (HPS) is an immune dysregulation characterized by cytokine storm and inflammation due to the uncontrolled proliferation of lymphocytes and macrophages. It is a rare life-threatening disorder that can be either primary due to a genetic defect, or secondary to infections, drugs, autoimmune disorders or malignancies.

METHODS

The diagnosis is established by the presence of five of the eight diagnostic criteria currently accepted by the Histiocyte Society (HLH-2004): fever; splenomegaly; cytopenias; hypertriglyceridemia and/or hypofibrinogenemia; hemophagocytosis in bone marrow, lymph nodes or spleen; decreased NK cell activity; ferritin > 500 µg/L; elevated CD25s.
A 30-year-old woman, with no known comorbidities, came to the emergency room with fever, asthenia, anorexia and vomiting. Examination revealed axillary adenopathy and hepatosplenomegaly.

RESULTS

Initial blood work showed LDH 537 U/L [208-378]; ferritin 3348 ng/mL [10-120]; triglycerides 562 mg/dL [30-200]; and CRP 3,93 mg/dL [0-0,5]. The hemogram revealed a hemoglobin of 8,7 g/dL [12-16], in addition to bicytopenia with leukopenia, $2{,}7x10^3$/µL [$4.2\text{-}10.5x10^3$] and thrombopenia, $108x10^3$/µL [$130\text{-}450x10^3$].
Microbiological analysis ruled out infection by leishmania, toxoplasmosis, cytomegalovirus, Epstein-Barr virus, parvovirus, SARS-Cov2 by serology tests and various bacterial infections because of negative bacterial culture. Also, autoimmune analysis (antinuclear antibodies and antibodies against double-stranded DNA) was negative.
Bone marrow aspirate and immunophenotyping were performed, concluding that the three cell lines were well represented, without signs of dysplasia. Hemophagocytosis is not observed. No lymphocyte alterations (2.5% NK cells). Therefore, the determination in serum of the soluble receptor CD25 was requested, resulting in a positive result of 1821 U/mL [158-623].
Finally, a biopsy of skin lesions revealed coagulative necrosis with abundant karyorrhexis remnants compatible with HPS secondary to Kikuchi-Fujimoto's disease, starting the immunosuppressive treatment with dexamethasone.

CONCLUSIONS

Due to the high mortality of HPS, it is important to establish an early diagnosis, which includes clinical and laboratory parameters.
In our case, the patient was diagnosed fulfilling 6 out of 8 HLH criteria, where the laboratory has a fundamental role in order to initiate the treatment to halt the hyperinflammatory process.

Haematology, including haemostasis

W038

UNUSUAL FINDING IN A GEL ELECTROPHORESIS: ABNORMAL MONOCLONAL PROTEIN

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BACKGROUND-AIM

Monoclonal gammopathies are a heterogeneous group of diseases in which the laboratory professional plays a leading role in the diagnosis, monitoring and prognosis. The knowledge about the limitations of the technologies used is important to improve their management.
Faced with a paraproteinemia study where, after carrying out gel electrophoresis, we observed an extremely thin band in the gamma zone and in immunofixation this same band appeared in all the evaluated chains. Although it looks like an artefact, we must rule out the presence of a monoclonal peak or interferents that can alter the electrophoresis.

METHODS

Given this finding, the laboratory must rule out the presence of different interferents: the presence of fibrinogen in the sample, hemolysis, lipemia, jaundice, elevated concentration of protein components (CRP, tumour markers, etc.), therapy with biological drugs, intervention with opaque contrasts, treatment with antibiotics or plasma expanders.
Having ruled out all these possible interferents, we decided to check whether it was an interferer due to the polymerization of immunoglobulins. To do this, we proceeded to dilute 200 uL of the sample with 20 uL of acetylcysteine 100 mg / mL in ampoules. Shake cold and let the sample rest for 15 minutes. We then proceeded to immigrate again.

RESULTS

After dissolving with acetylcysteine, a monoclonal peak was obtained consisting of M heavy chains and Kappa light chains, the fine line of the rest of the evaluated chains disappearing. Acetylcysteine is a mucolytic used to dissolve disulfide bridges in mucosal secretions, and it is also dissolved disulfide bridges formed in the polymerization of immunoglobulins.

CONCLUSIONS

Faced with the unusual finding of an anomalous band in the gel electrophoresis, the clinical laboratory must consider the possible interferents that can affect patient samples, either due to high protein components or treatments that can cause interference.
The laboratory must have all the means at its disposal to obtain a response to a clinical issue.

Haematology, including haemostasis

W039

LYMPHOCYTE POPULATION IN SARS-COV-2 INFECTION

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BACKGROUND-AIM

SARS-CoV-2 emerged into the human population in late 2019 causing the COVID-19 pandemic, which represents a major health crisis worldwide. Although the pathogenesis of the virus is not really well-known, recent publications conclude that SARS-CoV-2 causes lymphopenia. In the majority of the acute viral diseases we expect lymphocytosis with morphological changes in mononuclear cells.
Thus, the aim of this study is to compare changes in the lymphocyte population between different viral diseases.

METHODS

A total of 85 patients were included in our study of whom 45 patients had positive test result for SARS-CoV-2, 26 for mononucleosis viruses (MV) – including Epstein-Barr and Cytomegalovirus – and 14 for Human Immunodeficiency Virus (HIV). We performed cell blood count (CBC) and leukocyte differential and immunophenotype (IP) including CD3, CD4 and CD8 antibodies. CBC was performed by the Sysmex XN analyzer and IP by FC500 Beckman-Coulter. Absolute numbers of neutrophils, lymphocytes and monocytes as well as lymphocyte populations were analyzed by pairwaise comparison (SPSS software). CD4/CD8 ratio was also analyzed.

RESULTS

Significant differences were found between groups in all parameters tested. SARS-CoV-2 patients presented significant differences in absolute lymphocyte population (Mean=1.37x103/µL). Interestingly, SARS-CoV-2 patients presented a significant neutrophilia when compared with the other two groups. When the lymphocyte populations where specifically studied, it was observed that SARS-CoV-2 patients presented a significantly lower amount of CD3-positive lymphocytes. Finally neither significant differences were found in the CD4 population nor the CD4/CD8 ratio in all the groups studied.

CONCLUSIONS

In our study we concluded that SARS-CoV-2 patients presented lymphopenia when we compared this population to other viral infections. This observation could be related to the fact that in SARS-Cov-2 patients the amount of CD3 cells is clearly lower than in the other patients studied. These differences could be attributed to a lower amount of CD8+ lymphocytes. Although mean of CD4 population was different between SARS-CoV-2 and MV patients, when it comes to absolute number of cells detected by IP, there were no differences in the CD4/CD8 ratio.

Haematology, including haemostasis

W040

CHRONIC EOSINOPHILIC LEUKAEMIA IN A PATIENT WITH PROSTATE NEOPLASIA: RELEVANCE OF THE PERIPHERAL BLOOD FILM EXAMINATION

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BACKGROUND-AIM

Low cell blood counts may be found related to haematological or non-haematological diseases. The case presented herein illustrates the relevance of peripheral blood (PB) film examination for establishing the diagnosis.

METHODS

A 63-year-old man was admitted to the Emergency Department for fever secondary to urine infection. An automatic blood cell count showed low values of leukocytes (3.25x109/L), haemoglobin (111 g/L) and platelets (70x109/L). High levels of C-reactive protein (13.5 mg/dL; normal values (NV): <1 mg/dL), lactate dehydrogenase (926 U/L; NV: <234 U/L) and ferritin (11,654 ng/mL; NV: 200-400 ng/mL). PSA levels in serum were high (30 μg/L; NV: <3.5 μg/L). An ultrasound scan showed an enlarged prostate (weight: 40 g; normal: 20-25 g). A prostate tumour was suspected and a biopsy was demanded.

RESULTS

PB review showed high percentage of eosinophils (17%). They were hypogranulated, some of them with a high number of nuclear lobes. In addition, promyelocytes, myelocytes and metamyelocytes with eosinophilic granulation and 1% of blasts were seen. A bone marrow aspirate (BMA) was recommended with the diagnostic orientation of haematological disease.
BMA showed the presence of high number of eosinophils, together with eosinophilic hypogranulated precursors, showing aberrant (pre-eosinophilic) granulation. Immunophenotype revealed a 74% of abnormal cells (CD13+, CD33+, CD34-, CD117-, HLA-DR+, CD15+, CD11b+/-). Mutations or rearrangements in leukaemia-related genes were not found by cytogenetic studies. Nevertheless, next-generation sequencing (NGS) using a myeloid panel of 40 genes showed a type A mutation in NPM1 (c.863_864insTCTG) in PB. Two mutations in TET2 and one in SRSF2 were also detected. Diagnosis of chronic eosinophilic leukaemia was stablished.
Besides, the prostate biopsy revealed an adenocarcinoma. Radiotherapy was initiated and, when finished, the patient received intensive leukaemia chemotherapy.

CONCLUSIONS

Morphological abnormalities contributed to the myeloid neoplasm diagnosis in a patient in whom a simultaneous prostate adenocarcinoma was detected. This case illustrates the relevance of a careful PB smear examination for establishing the diagnosis of haematological diseases, even in the concurrence of confounding such a solid neoplasia.

Haematology, including haemostasis

W041

DIAGNOSTIC WORK-UP FOR THROMBOTIC MICROANGIOPATHY IN LIVER TRANSPLANT RECIPIENTS: CHALLENGING ADAMTS13 ACTIVITY ASSAYS.

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BACKGROUND-AIM

After liver transplantation (LTX), thrombotic microangiopathy (TMA) can occur, although rare, but life-threatening. ADAMTS13, which is predominantly produced in hepatic stellate cells, may be moderately decreased in severe liver disease. Severe ADAMTS13 deficiency leads to thrombotic thrombocytopenic purpura (TTP), a specific form of TMA. ADAMTS13 tests using the FRETS-VWF73 substrate may be confounded by high bilirubin concentrations and give falsely low values.
Aims: How many LTX patients develop TMA? Result comparison of ADAMTS13 activity with different methods.

METHODS

25 consecutive LTX patients at our center between April and December 2017 were assessed before, 2-4 days, 7-9 days and during the first outpatient visit (median 14 weeks) after liver transplantation. In addition to routine liver transplant tests, a laboratory "TMA panel" with two different measurements of ADAMTS13 activity, ADAMTS13 antigen, haemoglobin, haptoglobin, LDH and schistocytes was performed.

RESULTS

Three patients showed signs of a TMA (platelets <100/nl, anemia, haptoglobin <0.35g/l, increased LDH, schistocytes >5‰). In 16 patients a moderately decreased ADAMTS13 antigen level as well as a moderately decreased ADAMTS13 activity (median 43.9% [min 22.7, IQR 37.0-46.0, max 49.1]) was detected, after LTX. All surviving patients had normal ADAMST13 activity with both assays [median 82%] and antigen values [median 0.8ng/ml] at the first outpatient visit. In patients with normal or moderately elevated bilirubin, there are no differences in ADAMTS13 activity between the two assays (median FRETS-VWF73 48.0% vs. chromogenic 55.3%). Significant differences were detected at bilirubin levels >4mg/dl (FRETS-VWF73: median 30.5 [min. 5.3, IQR 20.3-41.7, max. 80.5], chromogenic assay: median 52.2% [min. 28.3, IQR 46.2-58.9, max. >107.0]. Using FRETS-VWF73, 4 of 25 LTX patients had ADAMTS13 activity <15%, two of them <10%, leading to misdiagnosis of TTP.

CONCLUSIONS

The occurrence of TMA should be considered in LTX patients with decreased platelets and haemolytic anaemia and ADAMTS13 activity should be analysed to exclude TTP. We confirm that severely elevated bilirubin levels lead to falsely decreased ADAMTS13 activity and therefore not to rely on the FRETS-VWF73assay in these situations.

Haematology, including haemostasis

W042

SAMPLE STABILITY FOR ANTI-XA TESTING ON THE SYSMEX CN-6000

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BACKGROUND-AIM

Background: Patients receiving unfractionated heparin (UFH) therapy require monitoring of anti-Xa activity to ensure adequate anticoagulation. The release of platelet factor 4 (PF-4) from platelets over time has a neutralising effect on anti-Xa activity, leading to falsely reduced results and potential over-anticoagulation. Local guidelines accept requests up to 4 hours post venepuncture, and limit add on requests to this time period. An increase in add on requests during the COVID-19 pandemic and receipt of new coagulation analysers prompted review of these recommendations.
Aim: To assess the stability of Anti-Xa activity in Sodium Citrate samples from patients treated with intravenous UFH.

METHODS

Samples from ITU patients on UFH were hand delivered to the laboratory, they were confirmed as having met pre-analytical requirements, centrifuged and tested following routine protocols. Anti-Xa testing (Hyphen Heparin LRT kit) and APTT ratio (Siemens Actin FS) were tested on the Sysmex CN-6000 analyser.
After analysis, 18 samples were mixed and kept at room temperature before repeat centrifugation and analysis at 30 minute intervals up to 4 hours, with the aim of replicating the whole blood environment.
Additionally, XX samples were centrifuged upon arrival in the laboratory and tested at the same timed intervals, from the primary tube, with the aim of replicating add on requests to stored plasma.

RESULTS

Anti-Xa results upon initial testing ranged from 0.1-1.5 iu/mL. For samples stored as whole blood, the mean anti-Xa results decreased to 82% of the initial result after 2 hours, decreasing further to 75% after 4 hours. APTTr results >2 showed more significant decrease, and those with an APTTr ≤2 appeared to be stable over the 4 hour testing period. For samples stored as plasma in the primary tube stability over 6 hours was confirmed.

CONCLUSIONS

Anti-Xa and APTTr results decreased significantly in whole blood, with higher results showing greater effects of time. Local policy has been amended to shorten the acceptance time window post venepunture for patients on UFH. However the time window for addition of anti-Xa to samples already received by the laboratory has been extended.

Haematology, including haemostasis

W043

RED CELL DISTRIBUTION WIDTH AS A PROGNOSTIC FACTOR IN PATIENTS WITH HEPATOCELLULAR CARCINOMA

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BACKGROUND-AIM

The Platelet Distribution Width (PDW) indicates the platelet activation and can be altered in malignancies

METHODS

48 patients with liver disease, 17 women and 31 men (median age: 62.2±13.7) and 20 healthy individuals were included. According to the disease progression, the study groups were: group I concerning patients with chronic active hepatitis B and C, group II concerning patients with liver cirrhosis and group III concerning patients with HCC. Platelets number, Mean Platelet Volume (MPV) and PDW were determined by Sysmex XE 2100. Data analysis took place using the SPSS v.19.0. One way ANOVA test (post hoc tests-Bonferroni) was applied. Results were attributed as mean value ± standard deviation. P value of <0,5 was considered to be statistically significant.

RESULTS

PLTs value was 194.32±57.14-K/μl in group I, 181.75±122.28-K/μl in II and 134.40±43.54-K/μl in III, significantly lower in all groups than the control group (272.20±45.47-K/μl, p<0.05) with statistically significant difference between I and III (p=0.031). The MPV value was 10.78±0.86 fl in I, 11.52±0.99-fl in II, 11.86±1.00-fl in III, significantly higher in II και III than the control group (10.18±0.47-fl, p<0.05) with statistically significant difference between I and III (p=0.001). PDW value was 13.31±2.04 fl in I, 14.47±2.64-fl in II, 16.07±3.46-fl in III, significantly higher in III than the control group (11.76±1.04-fl, p=0.002) with statistically significant difference between I and III (p=0.023).

CONCLUSIONS

PDW could be a useful estimation tool of liver disease progression in patients with HCC.

Haematology, including haemostasis

W044

CLUSTERING OF MATURE LYMPHOID PATHOLOGIES USING MORPHOMETRIC PARAMETERS AND UNSUPERVISED MACHINE LEARNING

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BACKGROUND-AIM

Cell blood count (CBC) and leukocyte differential are part of the screening for reactive and neoplastic haematological pathologies. Cell population data are morphometric parameters that measure the characteristics of nucleated cells in order to group and differentiate between them and also to generate qualitative screening alarms. The aim of this study is to differentiate between reactive and clonal lymphocytosis using morphometric parameters integrated into a clustering algorithm.

METHODS

Absolute lymphocyte count and cell population data obtained with the Beckman Coulter DxH900 analyser from 183 patients were retrospectively obtained. Data for the seven morphometric parameters were presented as mean and standard deviation. The uniform manifold approximation and projection for dimension reduction (UMAP) method was used to reduce the dimensionality and cases were grouped using the density-based spatial clustering of applications with noise (DBSCAN) algorithm.

RESULTS

The 183 patients were studied at diagnosis and classified into 5 groups when determining the cause of lymphocytosis: 58 reactive lymphocytosis, further divided into 24 infectious mononucleosis caused by Epstein Barr virus or Cytomegalovirus and 34 by other causes, 83 chronic lymphocytic leukaemia, 23 mature B-cell neoplasm different than CLL and 19 mature T and NK neoplasm. A total of 297 patients without lymphocytosis were used as reference.
Dimensionality was reduced using the UMAP technique (hyperparameters: min_distance=0.4, neighbours=7) and cases were clustered using DBSCAN (hyperparameters: eps=1.48, min_samples=42). Four clusters and 112 non-clustered points were obtained. 94% of the reference population was clustered in the first three clusters. 85% of non-infectious reactive lymphocytosis were classified in cluster 1 and 2. 94% of mature T-cell lymphoid neoplasms were classified in cluster 1. The majority of mature B-cell lymphoid neoplasms and CLL were not assigned to any cluster making them 82% of the outliers.

CONCLUSIONS

Mature lymphoid neoplasms are very heterogeneous in terms of phenotypic expression and clinical course. Morphometric parameters together with machine learning algorithms can detect cases with atypical lymphoid population with predominance of T, NK or B cells.

Haematology, including haemostasis

W045

ASSESSMENT OF UNICEL DXH-900 CELL MORPHOLOGY PARAMETERS ON NEUTROPHILS FOR EARLY DIAGNOSIS OF MYELODYSPLASTIC SYNDROMES

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BACKGROUND-AIM

Recognition of cytological myelodysplasia (CMD) is the cornerstone of diagnosis, classification and prognosis of myelodysplastic syndromes (MDS). The characterization of CMD has shown moderate reproducibility among observers. Currently, Haematologic Analyzers provide magnitudes named Cell Morphology Parameter (CMP); which can improve the MDS diagnosis.
The aim of this study was to evaluate the correlation between CMPs provided by the analyzer DxH-900 (Beckman Coulter) and the grade of CMD in neutrophils populations, and their diagnostic performance in the prediction of MDS.

METHODS

Peripheral blood (PB) of 57 MDS patients (diagnosis based on combination of morphological features on PB and bone marrow, cytogenetic and molecular data) were selected for the SMD Group. 71 healthy patients were selected for the Control Group.
PB smears were reviewed by 2 experienced observers,%hypogranulated neutrophils (%HYPOG) and %hyposegmented neutrophils (%HYPOS) were quantified through digital microscopy (Cellavision). Differences among observers >10% were corrected with a third observer. %HIPOG or %HIPOS >10% were considered significant.
CMPs were obtained from the haemogram processed on DxH-900. This analyzer uses the VCS technology to measure neutrophils' CMPs based on Volume (MN-V-NE and SD-V-NE), Conductivity (MN-C-NE and SD-C-NE) and Laser Scatter in different angles (MN-SM-NE and SD-SM-NE (9^o-43^o); MN-SU-NE and SD-SU-NE (9^o-19^o); MN-SL-NE and SD-SU-NE (20^o-43^o); MN-SA-NE and SD-SA-NE (5^o); MN-AL2-NE and SD-AL2-NE (0^o)).
Quantitative variables are expressed as medians (interquartilic range). Differences between groups with U Mann-Whitney test. Associations among groups with Spearman correlation. Diagnostic performance studied with ROC analysis (AUC>0.8). A p value<0.05 was considered significant. Program SPSS software (v25.0).

RESULTS

Differences between Control Group and MDS Group were found in all CMPs except MN-V-NE.
%HYPOG inversely correlated with Scatter CMPs MN-SM-NE (r: -0.362) and MN-SA-NE (r:-0.360) strongly (p<0.01) than MN-SU-NE (r:-0.308) and MN-SL-NE (r:-0.326)(p<0.05).
%HYPOS inversely correlated with Conductivity CMP MN-C-NE (r:-0.436; p<0.01) and Scatter CMPs MN-SM-NE (r: -0.345) strongly (p<0.01) than MN-SU-NE (r:-0.333), MN-SL-NE (r:-0.299) and MN-SA-NE (r:-0.283)(p<0.05).%HYPOG showed stronger correlation with Scatter CMPs than %HIPOS.
%HYPOG>10 expressed significant differences when compared to %HYPOG<10 on MN-SM-NE, MN-SU-NE, MN-SL-NE, MN-SA-NE; p<0.05. Meanwhile %HYPOS>10 expressed significant differences with %HYPOS<10 on MN-C-NE, MN-SM-NE, MN-SU-NE and MN-SL-NE; p<0.05.
AUC of CMPs MN-C-NE [AUN=0.828 (CI95%:0.747-0.910)], MN-SM-NE [AUC=0.816 (CI95%:0.742-0.890)], MN-SU-NE [AUC=0.828(CI95%:0.757-0.899)], SD-V-NE [AUC=0.906 (CI95%:0.847-0.966)], SD-SM-NE [AUC:0.873 (CI %:0.812-0.935)], SD-SU-NE [AUC=0.971 (CI95%:0.947-0.995)] and SD-AL2-NE [AUC=0.867 (CI95%:0.803-0.932)] (p <0.001).

CONCLUSIONS

Scatter CMPs are related to cellular complexity and had stronger correlation with the grade of neutrofils' hipogranulation whereas Conductivity CMP correlates better with hyposegmented neutrophils. MN-C-NE
MN-SM-NE, MN-SU-NE, SD-V-NE, SD-SM-NE, SD-SU-NE and SD-AL2-NE presented an excellent performance diagnosis on recognition of CMD in MDS. CMPs implementation on routine testing can be usefull on early diagnosis of MDS.

Haematology, including haemostasis

W046

IMPROVED PREOPERATIVE IRON STATUS ASSESSMENT BY BIOCHEMICAL AND NEW HEMATIMETRIC PARAMETERS

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BACKGROUND-AIM

Iron deficiency anemia (IDA) is common in the preoperative period, being associated with poor outcomes. Guidelines recommend investigating patients with hemoglobin (Hb) values < 130 g/L requiring high bleeding risk surgery or a strong likelihood of blood transfusion. The WHO recommends ferritin (F) as the primary measure of iron status. However, in patients with inflammation, elevated serum F and IDA may coexist. The aim of this study is to improve IDA diagnosis in surgical patients by combining new hematimetric parameters and biochemical tests.

METHODS

575 patients scheduled for knee or hip replacement surgery were included retrospectively. The variables considered were: age, gender, F, transferrin, transferrin saturation (TSAT), CRP, creatinine, Hb, reticulocyte Hb content (CHr), reticulocyte mean corpuscular Hb content (MCHr), hypochromic red cells (HYPO), and hypochromic reticulocytes (HYPOr). Inflammation was defined as CRP>1 mg/dL. Patients were classified following the algorithm included in Perioperative management of anemia and iron deficiency consensus. Given the imbalanced number of patients with anemia (N=100) and without (N=475), synthetic data was generated by randomly oversampling. The data set was split into training and test set before performing multivariate statistical analysis through logistic regression (LR), and classification tree (CT) through the CART algorithm considering the occurrence of IDA as the response variable. Statistical analyses were performed using R.

RESULTS

A total of 100 patients presented Hb levels below 130 g/L. High CRP values were found in 87. CT model showed accuracy, sensitivity and specificity values of 86%, 91% and 81%, respectively (AUC of 87%) to distinguish patients with IDA or without anemia. Among all the explanatory variables considered initially, only gender (OR for female: 3.58), creatinine (OR:1.34), HYPO (OR:1.29), HYPOr (OR:1.06), F (OR:0.97), CRP (OR:1.23), TSAT (OR:0.81) and transferrin (OR:4.16) were early independent predictors of IDA by LR, showing accuracy, sensitivity, and specificity values of 81%, 84% and 77%, respectively (AUC of 89%).

CONCLUSIONS

The proposed models overcome the limited ability of F to detect iron deficiency when inflammation is present, showing a good performance to early predict IDA in surgical patients.

Haematology, including haemostasis

W047

COMPARISON OF UNDILUTED AND DILUTED FIBRINOGEN IN CONCENTRATION AREA BETWEEN 4.5 AND 7 G/L

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BACKGROUND-AIM

Measuring fibrinogen concentration is important for blood coagulation disorders. According to the manufacturer (Siemens, Germany), fibrinogen concentration above 4.5 g/L should be diluted (1:4), but the concentration of the highest calibrator is always over 7 g/L (more precisely 7.71 g/L on used calibration curve). We used commercial reagent Siemens Dade Thrombin (Siemens, Germany) to determine fibrinogen in citrate plasma (3.2% sodium citrate) on coagulation analyzer Sysmex CS2500 (Japan) in the Department of Medical Biochemistry and Laboratory Medicine, Merkur University Hospital. The aim of our study was to compare fibrinogen concentrations in undiluted and diluted plasma in the area between 4.5 and 7 g/L.

METHODS

Analytical method (modified Clauss assay) is accredited according to ISO 15189 and controlled by an external quality assurance (EQA) program of an independent organizer ECAT (Netherlands). We performed comparison on 29 patient plasmas who had initial fibrinogen concentration between 4.5 and 7 g/L. Dilution 1:4 was made automatically immediately after first undiluted measurement using Dade Owren's Veronal Buffer (Siemens, Germany). For statistical significance we used Wilcoxon test (paired samples) because the data wasn't distributed normally (Kolmogorov-Smirnov test). Results were also compared using Passing-Bablok regression and Bland-Altman plot. RCV (reference change value) was calculated using a desirable intraindividual biological variation obtained from Westgard and analytical variation from long-term results of internal quality controls.

RESULTS

All the diluted results were lower than undiluted. Mean bias was -7.46% (-2,30% - (-18.75%)). Our null hypothesis was that there is no significant difference between two measures and the obtained p-value was $P < 0.0001$. Passing-Bablok analysis didn’t show any constant or proportional difference (y=-0.52(-1.47-0.53) + 1.03(0.82-1.21)). Bland-Altman analysis showed a mean difference -8.3%, which is lower than the total analytical error (13.6%). Calculated RCV value was 34.11%.

CONCLUSIONS

Our data show that the mean bias -7.46% is statistically significant ($P < 0.0001$), but not clinically because it is lower than the calculated RCV (34.11%). We conclude that dilution up to 7 g/L is not required.

Haematology, including haemostasis

W048

COMMUNICATION CIRCUIT OF PREOPERATIVE ANEMIAS IN SURGERIES WITH HIGH RISK OF BLEEDING

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BACKGROUND-AIM

The prevalence of preoperative anemia in major surgery is around 30%, varying depending on the type of surgery. Preoperative anemia, even moderate, carries high morbidity and mortality, which can be reduced by establishing protocols for the detection and correction of preoperative anemia prior to surgeries with a high risk of bleeding.
Patients scheduled for potentially bleeding surgeries (expected blood loss> 500 mL) are the greatest beneficiaries of these protocols. The improvement of hemoglobin should be considered in any patient with moderate-severe anemia undergoing major surgery, investigating the presence of preoperative anemia at least thirty days before the intervention, in order to make a differential diagnosis and establish the appropriate treatment when proceed.
From the Clinical Laboratory we implement a communication circuit for preoperative anemia in surgeries with a high risk of bleeding performed in our hospital centers (knee or hip arthroplasties and hysterectomies); developing a detection and alert system by the Biotechnology laboratory, who acts as a gatekeeper in the process, detecting and studying the type of anemia, warning and studying the type of anemia, and establishing, together with Hematology and the surgical service involved, the more appropriate treatment, in order to improve hemoglobin levels prior to surgery, thus minimizing perioperative anemia.
The aim of this study was to evaluate the clinical impact of the preoperative anemia communication protocol in surgeries with a high risk of bleeding, assessing the involvement of the clinical laboratory in the detection of cases susceptible to study and treatment. Review the treatments used to achieve the reduction of anemia prior to intervention, as well as the recovery of hemoglobin achieved.

METHODS

Retrospective observational study of preoperative anemia detected and reported during 2020.
Considering anemia hemoglobin figures <13 g / dl in both men and non-pregnant women, we made 30 reports of anemia out of 95 preoperative procedures performed for surgeries with a high risk of bleeding.
We analyzed the preoperative hemoglobin (Hb) data, as well as the necessary analytical data (ferritin, transferrin saturation index, folic acid, vitamin B12) to catalog anemia and establish specific treatment. Postoperative hemoglobin was determined (in 25 of the 30 patients), as well as hemoglobin in the analysis prior to surgery (preoperative hemoglobin) and after the establishment of specific corrective treatment (in 12 of the cases).

RESULTS

We detected anemia in the preoperative study with high risk criteria for bleeding in 31.6% of the cases (30 preoperative anemias out of 95 potentially bleeding surgeries performed), notifying and evaluating the specific treatment in 100% of the cases.
70% of the anemias detected corresponded to knee arthroplasty, 10% to hip arthroplasty, 10% to hysterectomy, and the remaining 10% to other potentially bleeding surgeries (hip fractures, removal of the femur nail).
Preoperative anemias were detected in patients aged between 44 and 95 years, with a median of 75 years, 83.3% of cases being women.
Based on the anemia study, treatment was established prior to surgery in all cases, recording the treatment of 18 of them: 10 patients received iron sucrose (600 mg), administered in one of the cases together with folic acid; 8 oral iron (of which 2 with folic acid, 1 with vitamin B12).
We analyzed the preoperative Hb and postoperative Hb data in 25 of the 30 patients (5 were excluded due to lack of any of the data). In 12 of these cases, hemoglobin was also determined in laboratory tests prior to surgery and after the introduction of specific corrective treatment (pre-surgical Hb).
In all the cases studied, the detection and warning of preoperative anemia triggered the study, characterization and treatment of the anemia prior to performing the surgical intervention.
A theoretical loss of 1.7 g / dl (median) of hemoglobin was observed after surgery in the cases studied (n = 25), although in patients with Hb control before the intervention after at least one month of treatment (n = 12) there was a reduction in anemia after treatment of 0.6 g / dl (median), and a real loss of Hb of 2.3 g / dl (based on preoperative Hb), which reflects more reliable post-surgery anemia.

CONCLUSIONS

We have managed to detect and report all preoperative anemia from surgeries with a high risk of bleeding during 2020, with the consequent study, characterization and treatment of anemia prior to surgery, with significant involvement and management of the process by the laboratory .
In order to optimize the measure of hemoglobin recovery prior to surgery, the analysis of pre-surgical hemoglobin (hemoglobin after the detection and treatment of anemia) is important to make a better interpretation of the data.

The patients who received treatment had milder postoperative anemia, starting with a higher hemoglobin level before the intervention, so their clinical situation is more advantageous after surgery than that which would be expected without preoperative anemia treatment.
Therefore, it is deduced that the communication and management of the study of preoperative anemia in surgeries with a high risk of bleeding as a warning value by the clinical laboratory has a very favorable clinical impact on the patient.

Haematology, including haemostasis

W049

INCIDENTAL DETECTION OF HEMOGLOBIN VARIANTS DURING CAPILLARY ELECTROPHORESIS ANALYSIS OF HBA1C

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BACKGROUND-AIM

Several hundred hemoglobin variants have been documented. The aim is to carry out a descriptive study of the hemoglobin variants found when performing the HbA1c determination for glycaemic control.

METHODS

50,044 patient samples (56.9% women; 42.6% men; 0.5% unknown) were analysed for the determination of HbA1c for glycaemic control from April 19 to July 13, 2021. The samples were processed by capillary electrophoresis (CE) (Capillarys 3, SEBIA®, France).

RESULTS

65 hemoglobin variants were found as incidental finding (65.2% women; 31.8% men), being 83% β-chain variants and 17% α-chain variants. It represents a frequency of 0.130% in the population studied. Delta chain variants were not taken into account.
15 different hemoglobins variants were found. Specifically, the percentages were 34.8% Hb S (NM_000518.5(HBB):c.20A>T (p.Glu7Val)), 13.6% of Hb Lepore (NM_000518.4(HBB):c.-7305_92+16del), 9.1% of Hb D Punjab (NM_000518.4(HBB):c.364G>C (p.Glu122Gln)), 7.6% of Hb C (NM_000518.4(HBB):c.19G>A (p.Glu7Lys)), 7.6% of J Paris, 6.1% of Hb Porto Alegre (NM_000518.4(HBB):c.29C>G (p.Ser10Cys), 4.5% of Hb J, 3% of Hb O-Arab, 3% of Hb J Baltimore, 3% of Hb Hopking. We only found a case of N Baltimore, Hb Nouakchott, Hb H, Hb E (NM_000518.5(HBB):c.79G>A (p.Glu27Lys)) and Hb Arya or Hb Savaria.
Hemogram alteration were found in 7 of the 15 variants types hemoglobins, understanding alterations in the hemogram as modifications in MCV, MCH or MCHC

CONCLUSIONS

Capillary electrophoresis is a method for determining hemoglobin variants that has been used for a relatively few years and has a number of advantages over HPLC. Fifteen types of hemoglobin variants were found, but HPLC is only able to detect nine of them.
On the other hand, the percentage of variants found in women was higher than in men. This is because the requests percentage is also higher in them due to the higher prevalence of diabetes in women.
The notable increase in the prevalence of hemoglobinopathies in our environment forces clinical laboratories to have different tools suitable for both determination, HbA1c and diagnosis of hemoglobinopathies.
Moreover, capillary electrophoresis, it is an automated, precise and high-performance method that also allows the separation of the most common hemoglobins variants, as well as their quantification.

Haematology, including haemostasis

W050

THE EVALUATION OF VARIOUS HEMATOCRIT VALUE SAMPLES ON YUMIZEN SPS AND DXH SLIDEMAKER STAINER II (SMS II) AUTOMATED SYSTEMS.

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BACKGROUND-AIM

High end laboratories are in the need of automated slide maker and stainer due to high pressure on turn around time for each sample. Nowadays various automated slide makers and stainer are available for the diagnostic laboratories commercially and they propose various technical arguments. The good peripheral smear slides is important to identify the blood cells those are crucial to identify pathologies. The aim of the study is to evaluate the quality of the slides produced by Yumizen SPS (HORIBA Medical, France) which does not change its spreading parameters, versus DxH Slidemaker Stainer II (SMS II) (Beckman coulter Inc, USA) which modifies its spreading parameters according to the hematocrit value.

METHODS

20 samples were processed of various hematocrit values on hematology analyzers Yumizen H2500 (HORIBA Medical, France) and DxH 900 (Beckman coulter Inc, USA) followed by the automated slide maker and stainers of both companies as a reflex action.
The following protocol was set on both company's slide maker and stainers analyzer. 2 minutes wright, 2 minutes wright giemsa, 6 min wright giemsa buffer, 1 min distilled water and 2 min distilled water and then drying of the sample. These slides were reviewed by Dr Sukesh Nair under the microscope at the laboratory and pictures of the cells are digitalized for the review.

RESULTS

The results were studied mainly macroscopically and microscopically. The macroscopic view was a criteria to understand the good length of the smear. Under the microscope, the location of erythrocyte monolayer and coloration of the cells was important to review the cell morphology and its recognition. The complete poster will have images of the results obtained in laboratory.

CONCLUSIONS

The study concludes that smear quality of slides is independent from hematocrit value of patient on both the analyzers. Both the analyzers were able to give almost similar slides those were examined macroscopically and microscopically. The digitalized cell images shows more clarity on slides coming from Yumizen SPS. It should be noted that regularity of the smear length as well as of the area of the erythrocyte monolayer also allows a reading with a digitalizer such as Cellavision.

Haematology, including haemostasis

W051

INTEGRATED DIAGNOSIS OF THALASSEMIE INTERMEDIA IN THE LABORATORY

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BACKGROUND-AIM

Thalassemias are hemoglobinopathies due to reduced production of certain globin chains. The most frecuency are β and α thalassemias, which constitute Hb A, mainly expressed in postnatal life. Structural variants or hemoglobinopathies are also included in thalassemias, in which there is also a decrease in the synthesis of this abnormal chain. Thalassemias α0 or β0 occur when the altered allele does not encode any chain, and α + or β + when there is a decrease in synthesis of the altered allele. Clinically, thalassemia intermedia constitutes a highly variable group of symptomatic thalassemias, with a somewhat higher degree of anemia than the thalassemic trait but somewhat lower than thalassemia major, with occasional transfusion needs. Most thalassemias can be diagnosed by hemathologic analysis, morphologic data, and Hb electrophoresis and/or cromatography, but sometimes is important to use molecular techniques to establish the definitive diagnosis.

METHODS

We highlight two cases of hemoglobinopathies seen in the Vall d'Hebron Hospital. Along with the collection of clinical data in the anamnesis, analytical tests were performed in the laboratory. Blood analysis was performed using an automated cell counter (Sysmex XS-1000i®), and comprised of a red blood cell count (RBC), Hemoglobin (Hb), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), and Red Cell Distribution Width (RDW). And biochemical parameters were analized wirth Cobas® 8000 series Automatic Chemistry Analyzer (Roche Diagnostics). Cell morphology was observed on the blood smear and Brilliant Cresyl Blue stained red cells.
A routine screening for sickle cell disease (SCD) was performed by High Performance Liquid Chromatography (HPLC) cation exchange in the automated analyzers Variant II Turbo, Bio-Rad Laboratories ®, and capillary electrophoresis (Minicap de Sebia®). And the molecular diagnosis was carried out by different methods: polymerase chain reaction (PCR) and reverse hybridization which covers 21 α-globin prevalent mutations (α-Globin StripAssay®, ViennaLab Diagnostics GmbH) for the most common deletion mutations; multiplex ligation-dependent techniques probe amplification (MLPA), using the SALSA® MLPA® probemix P140-C1HBA (MRC-Holland, Amsterdam, Netherlands), or next generation sequencing (NGS) technologies for novel or non-common thalassaemia deletions; and DNA sequencing for non-deletion thalassaemia mutations (Sanger).

RESULTS

Two patients were included in the study.
First case described a neonatal screening for sickle cell disease in a 6-months-old girl with Pakistani consanguineous parents, carrying α-thalassaemia and examined the molecular causes underlying the family's phenotype. Physical examination showed no signs of haemolytic anaemia such as pallor, jaundice or o hepatosplenomegaly. The hematological analysis showed: a RBC 5.53X10^12/L, Hb 8.3g/dL, MCV 47.7fL and MCH 15pg, being the last three lowest than normal reference ones. There are no other cytopenia on the blood count, or significant changes in biochemistry with a normal iron profile. Routine screening for SCD by HPLC analysis and alkaline gel hemoglobin electrophoresis, demonstrated an abnormal band in Bart's retention time (RT) 10.6%, while Hb F, HbA1 and HbA2 were 8.9%, 69.9% and 1.5%. On the blood smear we found abnormal hemoglobin distribution with target cells, microcytes and poikilocytosis; and Brilliant cresyl bl ue staining showed unstable inclusion bodies. In the molecular study of Hb H disease detected a genotype not described to date (-α3.7 / --39.1), compatible with a heterozygous compound of -α3.7 deletion iherited from the mother combined with another wider deletion (--39.1) inherited from the father in the alpha cluster.
In the second case, a 38-year-old colombian man, resident in Spain two years ago, showed a clinical profile compatible with renal colic. The hematological analysis was compatible with a microcytic anemia: RBC 2.94x10^12/L, Hb 5.9g/dL, MCV 62.6fL, MCH 20.1pg, RDW 35.7%, and Reticulocits 30x10^9 / L. Biochemical parameters described hemolytic signs such as haptoglobin consumption <0.3g/L, elevated lactate dehydrogenase (LDH) 3600IU/L, deficit of folate <4.54ngmL, and iron overload with ferritin 562.9µg/L, transferrin 135mg/dL, transferrin saturation index 86%, iron 230µg/dL. Magnetic resonance imaging of the liver showed severe overload (m. Wood: 121.21 µ mol / g), and abdominal ultrasound a splenomegaly of 16.2 cm. A marked irregular distribution of hemoglobin, marked hypochromia with dyserythropoietic elements and fragments was observed in the peripheral blood smear. HPLC detected an increase in the concentration of HbF 57.3%, much higher than HbA2 9.2%. The reinforcement of the HbF band, a greater production of delta globin chains, to the detriment of beta chains was showed in electrophoresis alkaline and acidic. All of these data were highly suggestive of beta thalassemia of intermediate severity. Finally, a molecular analysis was performed by the Sanger sequencing technique of the complete Hemoglobin Beta (HBB) gene, in which two heterozygous variants were detected in the HBB gene: NM_000518.4: C.-79A> G in the promoter region, which produces a beta+ thalassemia phenotype; and NM_000518.4: c.316-2A> G, causing beta0 thalassemia.

CONCLUSIONS

It is important to carry out an integration between clinical observations and the laboratory analytical tests that allows a complete short-term diagnosis of thalassemies intermedias. So these will help to schedule a good treatment if necessary, and achieve a better prognosis.

Haematology, including haemostasis

W052

REVEALING THE INDIVIDUAL SENSITIVITY TO CHEMOTHERAPY OF CHILDREN WITH ACUTE LEUKEMIA

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BACKGROUND-AIM

In recent years, significant progress has been achieved in the treatment of childhood leukemias, up to a complete recovery. However the prognosis of the course of the disease is individual and depends on the individual characteristics of the body and the various sensitivity to current therapy. The attention of researchers is directed to the select the laboratory markers that help to identify the individual sensitivity of patients . It is known that the genetic status plays an important role in leukemia and reveals the toxic impact. Considering this fact, the aim of our work was to determine the sensitivity of patients to the genotoxic effects of chemotherapy using study the level of buccal micronuclei (MN) in dynamics.

METHODS

The yield of micronuclei in buccal cells was determined in dynamics. Cells were scraped off from the inner surface of the cheek, were evenly distributed on the slides, dried, fixed and were processed according Stich method. Light microscopy was used for the analysis of buccal cells for micronuclei (1000 cells per test). The data were processed using the statistical factorial dispersion analysis method (ANOVA).

RESULTS

20 children with acute leukemia were investigated. The number of micronuclei was determined at the beginning of treatment and at 15th and 33th days, because it is accepted that cytological indicators of these days reflect the severity of the process. Our data were equal to the clinical and laboratory data . All investigated patients showed the increase of level of MN during treatment, but using cluster analysis, 2 groups of patients were identified that differed in the intensity of the increase in frequencies of MN. The factorial analysis of variance revealed a statistically significant relationship between the rate of increased level of MN, their initial level and clinical-laboratory indicators.. These data illustrate differences in the sensitivity of individual patients to the genotoxic effects of chemotherapy detected by the MN method. Certainly , isolated method is not sufficient for prognosis the process and only a optimal complex of different tests is needed, although our studies have shown that used by us determining the level of buccal micronuclei method is quite informative,

CONCLUSIONS

Our studies have shown that the quite informative method of determining the frequencies of micronuclei in buccal cells is non-invasive and relatively simple. We recommend using this method for assessing the individual sensitivity of children with acute leukemia to the toxicity of chemotherapy to predict the course of the disease

Haematology, including haemostasis

W053

ALNET: A NEW DEEP LEARNING MODEL FOR THE DIAGNOSIS OF ACUTE LEUKAEMIA LINEAGE USING PERIPHERAL BLOOD CELL IMAGES

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BACKGROUND-AIM

Deep learning-based systems are increasingly becoming a part of clinical practise in hemopathology. The objective was to design and evaluate under real-case scenarios a CNN-based model to operate as follows: 1) the input was a set of images of an individual peripheral blood smear (PBS); 2) the outcome was the prediction of one of the following diagnosis: acute myeloid leukaemia (AML), acute promyelocytic leukaemia (APL), acute lymphoid leukaemia (ALL) or infection.

METHODS

We designed a new strategy with a two-step classification scheme (ALNet), where two separate classifiers worked in series. The first module consisted of a VGG16 trained to distinguish abnormal promyelocytes among lymphocytes, monocytes, reactive lymphocytes and blasts. The second module required a VGG19 to discriminate between myeloid blasts and B-lymphoblasts.
To evaluate ALNet in a clinical setting, we used an image dataset from three hospitals (Hospital Clínic de Barcelona, Josep Trueta and Germans Trias i Pujol), which contained a total of 4,424 cell images (1,358 abnormal promyelocytes, 1,096 myeloid blasts, 1,082 B-lymphoblasts, 459 reactive lymphocytes, 312 lymphocytes and 117 monocytes). PBS were stained with MGG and acquired by the CellaVision®DM96 analyser. To use the PBS as a diagnostic unit, a threshold was established, such that the diagnosis was predicted from the cell class with the percentage of images classified above this value.

RESULTS

Module 1 correctly classified 99.9% of lymphocytes, 97.6% of monocytes, 97.2% of reactive lymphocytes, 95.3% of abnormal promyelocytes and 92.8% of blasts. Moreover, 94.2% of images corresponding to myeloid blasts and 81.1% to B-lymphoblasts were correctly classified.
Regarding the predicted diagnosis by ALNet, sensitivity, specificity and precision values of 100% were obtained for all categories of module 1. Finally, 94.3% of the smears containing blasts were predicted as belonging to patients with AML and 86.2% to patients with ALL.

CONCLUSIONS

The multicentric study revealed ALNet is a predictive model designed with two serially connected CNNs to assist clinical pathologists in the diagnosis of acute leukaemia during the PBS review. It has been proved to distinguish neoplastic (leukaemia) and non-neoplastic (infections) diseases, as well as to recognise the leukaemia lineage.

Haematology, including haemostasis

W054

CORRELATIONS BETWEEN BIOMARKERS OF ACUTE MYELOID LEUKAEMIA BLASTS AND PARAMETERS OF AUXILIARY LABORATORY TESTS

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BACKGROUND-AIM

Acute myeloid leukaemia (AML) is a large group of blood and bone marrow malignant disorders characterised by the accumulation of undifferentiated myeloid lineage cells in peripheral blood or bone marrow. AML is diagnosed in approximately one third of all leukaemia cases and is responsible for almost 40% of deaths caused by leukaemia. The most inclusive classification of AML, suggested by The World Health Organisation (WHO), comprises blast morphology, cytogenetic, molecular genetic variations and immunophenotypic biomarkers. New biomarkers and insight into their expression are crucial for the expansion of the WHO classification, while connections between distinct types of blast immunophenotypes, blood count parameters, cytomorphology features and genetic testing results would carry significant clinical or prognostic value. The aim of the study was to evaluate associations between immunophenotypic markers of AML patients' blasts and other results of laboratory tests.

METHODS

Anonymised data of 95 patients with AML diagnosis was collected. This data was used to reveal associations between immunophenotypic data acquired by flow cytometry and the results of complete blood count, genetic blast mutations and morphological evaluation of cells. Patients were classified into two populations according to marker expression data: expression absent (CDX-)/ expression present (CDX+).

RESULTS

Quantitative parameters of AML patients' blood showed statistically significant associations with changes in blast biomarker expression: leukocytosis was associated with CD14+, CD15+, CD64+, CD99+, CD117-, CD133-, lymphocytosis - with CD36+ , CD133-, lymphopenia - with CD99-, CD117-, monocytosis - with CD7+, CD14+, CD15+, CD33+, CD36+, CD96+, CD117+, CD113+, basophilia - with CD14+.
Cytoplasmic granulation observed in cytomorphological testing had a statistically significant association with CD11a + and CD117+.
AML patients' mutations had statistically significant associations with biomarker expression: FLT3 mutation was associated with CD11b+, CD36+, CD64+, CD133-; NPM1 mutation - with CD14+, CD34-, CD117-; TP53 mutation - with CD33+; IDH1 mutation - with CD11b-, CD15-, CD96-; IDH2 mutation - with CD96-, HLA-DR-; CEBPA mutation- with CD64+, CD96+.

CONCLUSIONS

The obtained results showed that there are statistically significant associations between AML patients' blast immunophenotype and blood indices, cytomorphological and genetic test results.

Haematology, including haemostasis

W055

MULTICENTRE STUDY ON THE COMPARISON OF METHODS FOR THE MEASUREMENT OF ANTICOAGULANT ACTIVITY IN PATIENTS TREATED WITH DOAC (DIRECT ORAL ANTICOAGULANTS)

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BACKGROUND-AIM

Direct oral anticoagulants (DOACs) generally do not require dose adjustment based on laboratory results, but their monitoring is necessary in some clinical conditions. Methods include mass spectrometry (LC-MS / MS) (gold standard), functional tests (diluted thrombin time or dTT for dabigatran and chromogenic assay of anti-Xa activity for xabans), or more recently the thrombin generation test (TGT). In this work the results obtained with functional tests, LC-MS / MS and TGT for dabigatran (DABI) and apixaban (API) were compared.

METHODS

Samples of patients treated with DABI (n = 105) and API (n = 120) were collected in 7 hospitals and analyzed with the relative functional tests. The remaining aliquots were stored at -80 °C and subsequently transported to 2 other centers for analysis with LC-MS / MS and TGT.

RESULTS

LC-MS / MS and functional methods for DABI showed moderate correlation (rho = 0.699; p <0.001); the Passing-Bablok (PB) analysis showed a significant proportional systematic error (slope: 2.15; 95% CI 1.63-2.73). The correlations (n = 81) between the various TGT parameters and the values measured by the functional method or LC-MS / MS were 0.769 (p <0.001) and 0.721 (p <0.001) respectively for the lag phase, 0.690 (p <0.001) and 0.667 (p <0.001) for time to peak, -0.708 (p <0.001) and -0.611 (p <0.001) for peak height and -0.662 (p <0.001) and -0.527 (p <0.001) for ETP. Between the two methods for API, a high correlation was evident (rho = 0.871; p <0.001) and a significant proportional systematic error (slope: 0.74; 95% CI 0.69-0.80). The correlations (n = 77) between the various TGT parameters and the values measured by the functional method or LC-MS / MS were lower than those observed for DABI and equal respectively to 0.236 (p = 0.041) and 0.336 (p = 0.003) for the lag phase, 0.463 (p <0.001) and 0.493 (p <0.001) for the time to peak, -0.530 (p <0.001) and -0.499 (p <0.001) for the peak height. No significant correlation was present between functional method (-0.073; p = 0.534), or LC-MS / MS, and ETP (0.050; p = 0.665).

CONCLUSIONS

Important differences were found between functional methods and LC-MS / MS. Dabigatran correlates more than apixaban with the parameters of the TGT.

Haematology, including haemostasis

W056

ASSESSMENT OF THE ABSOLUTE NUMBER OF T-LYMPHOCYTES AND THEIR SUBPOPULATIONS IN CLL PATIENTS BEFORE AND DURING IBRUTINIB THERAPY.

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BACKGROUND-AIM

Infection complications are the main cause of mortality in chronic lymphocytic leukemia (CLL), and immunological dysfunction is one of the leading factors in their occurrence. The aim of this work is to assess the features of the subpopulation composition of T-lymphocytes (T-lym) (T-helpers (Th), cytotoxic T-lymphocytes (Tcyt), T regulatory cells (Treg), T-NK cells, naive Th, Th-memory, activated T-lym, TCR$\gamma\delta$ cells) in peripheral blood of patients with newly diagnosed CLL and receiving ibrutinib therapy.

METHODS

Hematological and immunophenotypic studies have been performed in 196 patients: 50 patients with previously untreated CLL (I group), 116 patients on ibrutinib therapy (group II) and 30 healthy donors. The subpopulation composition of T-lym (Th, Tcyt, Treg, T-NK, naive T-helpers, memory T-helpers, TCR$\gamma\delta$ cells, activated T-lym) was assessed on a flow cytometer (FACSCanto II (BD)) using a panel of monoclonal antibodies.

RESULTS

Compared to controls, all CLL samples were found to have higher the absolute number of T-lym ($p<0,0001$) by Th ($p<0,0001$) (especially of Th-memory ($p<0,0001$)), Tcyt ($p<0,0001$), Treg ($p=0,011$), TCR$\gamma\delta$ cells ($p=0,0003$), activated T-lym for both the CD3+CD25+ population (early activation) ($p<0.0001$) and CD3+HLA-DR+ population (late activation) ($p<0.0001$) in group I patients. In group II patients the absolute number of both T-lym ($p=0,0006$) and the absolute number of subpopulations of T-NK cells ($p=0,0007$) decreases compared to group I patients. At the same time, the values of the above subpopulations don't reliably differ from those of donors. Also, in group II patients have been marked by significant decrease of T-lym with markers of early and late activation as compared to group I ($p<0,0001$, but the values of these populations remain significantly higher than those of donors ($p=0,0007$- for CD3+CD25+ T-cells and $p=0,0079$ - CD3+HLA-DR+ T-cells).

CONCLUSIONS

CLL patients have been found to have quantitative and functional changes in the subpopulations of T-lym and NK cells, indicating dysregulation of the immune response, and a high risk of developing infections. Monitoring of immunological parameters for ibrutinib therapy make possible to estimate impact of ibrutinib on the adaptive anti-CLL immune response.

Haematology, including haemostasis

W057

COMPARATIVE ANALYSIS OF 2 METHODS OF FLOW CYTOMETRY FOR THE DETECTION OF MINIMAL RESIDUAL DISEASE IN CLL

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BACKGROUND-AIM

The introduction of targeted therapy for CLL leads to changes in the expression of individual antigens on the surface of B-lymphocytes and requires the search for new markers that maintain stable expression against the background of ongoing therapy to assess minimal residual disease (MRD).
Objective. Evaluate the expression of ROR-1 on B-lymphocytes during the course of CLL and the possibility of its use in the assessment MRD in CLL.
Compare the assessments results of MRD in CLL using two methods: standardized 8-color test (ERIC, 2016) and Dura Clone RE CLB Tube (BC) kit, which includes marker ROR-1.

METHODS

To evaluate ROR-1 marker expression on B-lymphocytes 107 samples were analyzed including 50 patients with primary CLL, 67 patients on therapy, 20 patients with reactive lymphocytosis and 30 donors. The study was performed on a FACS Canto II flow cytometer (BD).
64 bone marrow samples and 6 peripheral blood samples of CLL patients stabilized with K_2EDTA were analyzed for the evaluation of MRD. The study was performed on a flow cytometer Navious (Beckman Coulter) using a Dura Clone RE CLB Tube kit (BC). The reference method was the international standardized protocol (ERIC) for flow cytometry including light chains κ and λ types.

RESULTS

Among donors and patients with reactive lymphocytosis the percentage of B-lymphocytes expressing ROR1, in donors was 0.07±0.02% (M±m), in patients with reactive lymphocytosis - 0.05±0.02%, while in CLL there was a high expression of this marker on most B-lymphocytes both in the disease debut (77.7±2.7%), and with the ongoing therapy (87.0±1.8%). At the same time, there was a high correlation of this marker with the number of tumor CD19+CD5+ B-cells (r=0.9159).
Parallel testing for residual CLL tumor clone by two methods showed a high correlation of the results (r=0.9997). The sensitivity of the DuraClone RE CLB Tube MRD detection method was 97.4%, and the specificity was 87.1%.

CONCLUSIONS

ROR-1 expression on tumor B-lymphocytes remains stable against the background of current therapy, which allows the use of this marker in the evaluation of MRD CLL. The advantage of using the DuraClone RE CLB Tube kit containing ROR-1 is standardization of the study, reduction of study time, detection of the CLL tumor clone without evaluation of light chain restriction.

Haematology, including haemostasis

W058

MYELODYSPLASTIC SYNDROMES, REFRACTORY ANEMIA WITH RING SIDEROBLASTS AS AN IMPORTANT ENTITY OF MYELODYSPLASTIC SYNDROMES

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BACKGROUND-AIM

Myelodysplastic syndromes are clinically heterogeneous disorders characterized by clonal hematopoiesis, impaired differentiation, peripheral-blood cytopenias, and a risk of progression to acute myeloid leukemia.
Objectives: To highlight the importance of Perls staining and morphology as an important step towards the diagnosis, classification and prognosis of MDS

METHODS

This study included 44 patients diagnosed with MDS at Tirana University Hospital Center "Mother Teresa" during a period from January 2018-October 2019. Myelograms of these patients were examined after Perls staining for ring soderoblasts.

RESULTS

The mean age was 68 years, with female sex predominance. Examination of 44 cases revealed the presence of ring sideroblasts in 3 patients, the presence of normoblast with siderotic granules: >5% perinuclear granules covering in ring form over 1/3 of the perimeter of the nucleus, accounting for >15 % of the erythroid lineage. Erythroid displasia was prevalent.

CONCLUSIONS

Morphology is an important step in orienting the diagnosis of myelodysplasia and as a first step in the MDS diagnostic algorithm. Perls staining is an important method in the diagnosis, classification and prognosis of RARS.

Haematology, including haemostasis

W059

CHILDREN WITH HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS

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BACKGROUND-AIM

Hemophagocytic lymphohistiocytosis (HLH) is an aggressive and life-threatening syndrome of excessive immune activation and dysregulation that most frequently affects infants from birth to 18 months of age. It is a rare disorder with an incidence of 1,2:1.000.000.

METHODS

11 year old patient who comes to Emergency Room with a fever more 72 hours > 40oC, progressive abdominal pain, severe deterioration and intermittent vomiting. On examination, located pain in right hemiabdomen, righ lower quadrant. Laboratory test, an elevation of Acute Phase Reactors (APR) with C-Reactive Protein 146 mg/l, Procalcitonin 34 ng/ml and D-dimer 1929 ng/ml was observed. Abdominal ultrasound, signs compatible with ileitis are observed. Serology tests for SARS-COVID-19 was performed, with positive IgG and negative IgM. Polymerase Chain Reaction (nasal exudate) was negative.

RESULTS

The patient was admitted to the Paediatric Intensive Care Unit (PICU) for haemodynamic control. After 24 hours, cardiac dysfunction was present, bilateral pulmonary edema, vasoactive support and non-invasive ventilation was required. Echocardiography showed an increase in the size of the coronary arteries, so Immunoglobulin iv was started at 2 g/kg with methylprednisolone at 2 mg/kg/24h due to suspicion of Kawasaki Syndrome with shock. Bone marrow biopsy for thrombocytopenia and anemia was performed and showed an increase of hematophagocytosis. The patient presented acute renal failure with high level of creatinine and proteinuria in nephrotic range, ionic alterations (hypernatremia, hypokalemia and hypocalcemia) and hypoalbuminemia with capillary leakage syndrome, requiring hemodynamic support and treatment with furosemide and potassium cannonate. Antibiotic treatment was prescribed for 7 days. The clinical evolution and laboratroy test was positive. After 18 days admitted she was finally discharged.

CONCLUSIONS

The majority of children with HLH have severe clinical presentations with evidence of multi-organ involvement and rapid clinical deterioration. The role of the laboratory in the prognosis and follow-up of these patients is very important.

Haematology, including haemostasis

W060

INVESTIGATION OF PLATELET FUNCTION AND THROMBIN ACTIVATED FIBRINOLYSIS INHIBITOR IN CHILDREN WITH CONGENITAL HEART DEFECTS

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BACKGROUND-AIM

Open-heart surgery is associated with the development of thrombotic complications which is associated with an increased morbidity and in-hospital mortality in children with congenital heart defects.

METHODS

In our work, we studied the indicators of platelet aggregation with ristocetin, thrombin, and adenosine diphosphate before surgery using the Multiplate®. The levels of thrombin activatable fibrinolysis inhibitor were studied (by Stago STA Compact Max® coagulation analyzer) at three points: before surgery, 24 and 72 hours after surgery. The study included children with congenital heart defects aged from birth to one year who underwent heart surgery with cardiopulmonary bypass.

RESULTS

43 patients were diagnosed with thrombosis in the postoperative period, which is 28.1%. The patients were divided into 2 groups: group 1 without thrombosis and group 2 with thrombosis. It was noted that the indices of aggregation with ristocetin, thrombin and adenosine diphosphate in group 2 were higher preoperatively, but the differences between the groups were not statistically significant ($p>0.05$). The levels of thrombin activatable fibrinolysis inhibitor significantly increased at point 3 of the study compared with preoperative values in both groups of patients (44 (35; 51) > 70.5 (48.5; 93.5)% in group 1 ($p<0.001$) and 45 (32; 61) > 70.0 (39.0; 91.0)% in group 2 ($p<0.013$).

CONCLUSIONS

Preoperative aggregation rates with ristocetin, thrombin, and adenosine diphosphate do not differ significantly between patients with and without thrombosis. The levels of thrombin activatable fibrinolysis inhibitor are significantly increased 72 hours after surgery, which indicates inhibition of the fibrinolytic system e in children with congenital heart disease operated on with cardiopulmonary bypass.