(1) DIAGNOSTIC IMPACT OF GENETICS AND EPIGENETICS IN ACUTE MYELOID LEUKEMIA
Daniel A. Arber, MD
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The diagnostic category of acute myeloid leukemia (AML) is actually a heterogeneous group of diseases that are now partially subclassified based on recurring cytogenetic abnormalities and the presence of specific gene mutations. Other cytogenetic and molecular genetic changes in AML impact the prognosis of these specific disease groups and are increasingly used to place patients into risk groups that may drive specific therapies. This presentation will review the more common genetic events in AML, including their impact on the epigenetics of this disease. The 2008 WHO classification includes a number of specific cytogenetic abnormalities as definitive for AML subtypes and these genetic abnormalities are felt to represent disease-initiating genetic changes. The most common of these include the t(15;17)q24.1;q21.1, resulting in the fusion of PML and RARA, in acute promyelocytic leukemia, the t(9;11) (p22;q23) involving the KMT2A (previously known as MLL) and translocation of the core binding factor (CBF) and acute promyelocytic leukemia with PML-RARA now has a favorable prognosis when treated with therapies that include all-trans retinoic acid or arsenic trioxide. The CBF AMLs include AML with (8;21) (q22;q22) RUNX1-RUNX1T1 and AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22) CBFB-MYH11. The RUNX1 and CBFB genes encode different components of the CBF protein, a critical protein in normal hematopoiesis. Disruption of the CBF protein inhibits binding of the protein to DNA targets and perturbs this normal process. Despite the difference in morphology for the two CBF AMLs, they have a similar, favorable prognosis with current therapeutic regimens. Other AML subtypes with recurring genetic abnormalities in the 2008 WHO classification include AML with t(6;9)(p23;q34) DEK-NUP214 and AML with inv(3)(q21q26.1) or t(3;3)(q21;q26.1), now known to result in a GATA2-EVI1 fusion, which are associated with myelodysplastic changes and a generally poor prognosis; and AML with t(1;22)(p13;q13) RBM15-MKL1 which is usually an infant leukemia with megakaryoblastic features. Although not included in the 2008 classification, AML with BCR-ABL1 is now considered a distinct entity if prior CML is excluded and is possibly amenable to therapy with tyrosine kinase inhibitors. Outside of the specific cytogenetic abnormalities listed above, other cytogenetic changes impact prognosis and disease classification in AML. Cytogenetic changes associated with myelodysplastic syndromes (MDS), most commonly complex abnormalities and deletions of chromosomes 5 and 7, are predictive of a poor prognosis and are used, along with other MDS-associated abnormalities, to support a diagnosis of AML with myelodysplasia-related changes (in the absence of prior cytotoxic therapy). In addition to specific cytogenetic translocations, various gene mutations also frequently occur in AML. Similar to karyotype abnormalities, some of these mutations are considered to be early, disease-defining events in AML and others occur across a spectrum of AML types and are considered prognostic markers. While initially studied as potential prognostic markers in AML with a normal karyotype, gene mutations are now often studied in a variety of settings that include testing in association with specific cytogenetic abnormalities. AML with mutated NPM1, the most common mutation in AML and AML with mutated CEBPA are provisional entities in the 2008 WHO classification and are generally associated with a favorable prognosis. These mutations do not usually occur with the recurring cytogenetic abnormalities discussed above. While usually associated with a normal karyotype, approximately 15% of cases will occur with a non-specific cytogenetic abnormality and this finding does not appear to impact the favorable prognosis of this general group. AML with mutated RUNX1 is also felt to represent a distinct disease group when occurring in de novo AML, although this mutation is associated with a generally poor prognosis. Most of the other mutations in AML are considered more as prognostic factors and do not define specific disease entities. These mutations may be acquired over time, or may be present very early, in pre-leukemic stem cells, but are often associated with other genetic abnormalities. FLT3 mutations are the second most common mutation in AML and may represent an internal tandem duplication (ITD) or, less commonly, a point mutation in the tyrosine kinase domain (TKD). In general, FLT3 mutations, especially ITD mutations, confer a worse prognosis in AML, but these mutations occur across a wide spectrum of AML subtype. While very common in AML with a normal karyotype, FLT3 mutations are also common in acute promyelocytic leukemia, AML with t(6;9)(p23;q34) and AML with mutated NPM1. In the latter case, the FLT3 mutation impacts prognosis by moving the case from a good prognostic group to a more intermediate-risk group. Mutations of RUNX1 are the third most common mutation in AML and also occur in conjunction with NPM1 and other mutations and tend to be associated with a worse prognosis. Mutations of KIT are, overall, relatively uncommon in AML, but are present in approximately 25% of CBFL-types of AML and are a poor prognostic indicator in these otherwise good prognosis disorders. Less common mutations in AML, include mutations of IDH1, IDH2, TET2, WT1, ASXL1, U2AF1 and TP53. While these abnormalities require further study, most have to date been shown to have an indeterminate or negative impact on prognosis, with TP53 mutations usually associated with complex cytogenetic abnormalities and a dismal prognosis. Epigenetic changes in AML include a variety of factors that include protein expression and alteration in DNA methylation. Both global DNA and gene specific methylation are reported to correlate with a poor prognosis, several specific gene mutations (DNMT3A, IDH1, IDH2 and TET2) alter normal gene methylation and hydroxymethylation pathways and these changes may be of more significance than performing complicated methylation studies. Similarly, while detection of increased expression of proteins such as BAALC and ERG are associated with a worse prognosis in AML, such studies are not routinely performed and are of unclear significance when paired with multi-gene panel mutation studies. In summary, the genetics of AML are complicated and the array of gene alterations in the disease may cause confusion on determining the optimal approach to the diagnosis of AML. While not all mutation studies are needed in all cases, the rapid development of next generation gene panels may make a step-wise approach to testing unnecessary.

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(2) HEPcidin and iron disorders: New biology and clinical approaches.
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Hepatic hormone hepcidin is a principal regulator of iron homeostasis and a pathogenic factor in common iron disorders. Hepcidin deficiency causes iron overload in hereditary hemochromatosis and iron-loading anemias, whereas hepcidin excess causes or contributes to the development of iron-restricted anemia in inflammatory diseases, infections, some cancers and chronic kidney disease. Because of this, hepcidin may become a useful tool for diagnosis and management of iron disorders. Furthermore, a number of strategies that target hepcidin, its receptor and its regulators are under development as novel therapeutic approaches for diseases associated with iron dysregulation.

References

(3) Telomere biology and the new telomeropathies.
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Telomeres, repeated short nucleotide sequences and associated shelterin proteins, constitute the termini of linear chromosomes. Telomeres protect chromosomes from recognition as double stranded DNA breaks. Telomeres shorten with every cell division, and attrition is partly abrogated by the telomerase repair complex. Critical telomere shortening accounts for the Hayflick phenomenon, and critically short telomeres cause genomic instability. Telomerase mutations are etiologic in the telomeropathies, organ failure syndromes that include bone marrow failure, pulmonary fibrosis, and liver disease. Telomere content of peripheral blood leukocytes can be measured by a variety of assays, some now commercially available. The phenotype and penetrance of telomeropathies is highly variable. Dyskeratosis congenita in childhood is associated with mucocutaneous findings, telomeropathies in adults with early hair graying. Mutations in TERT (the gene encoding the reverse transcriptase) cause liver disease, mutations in TERC (encoding the RNA template), pulmonary fibrosis. Accelerated telomere attrition is the major risk factor for malignant clonal evolution in SAA; chromosomal aberrations can be observed in tissue culture months to years preceding clinical MDS/AML. Sex hormones upregulate TERT expression, and danazol in a prospective NIH clinical protocol has been effective in improving blood counts in telomeropathy patients and elongating telomeres.

References
Gutierrez-Rodrigues F1, Santana-Lemos BA1, Scheucher PS1, Alves-Paiva RM1, Calado RT1. Direct comparison of flow-FISH and qPCR as diagnostic tests for telomere length measurement in humans. PLoS One. 2014 Nov 19;9(11):e113747
(4) PAST PRESENT AND FUTURE OF QUALITY IN HAEMOSTASIS TESTING

Steve Kitchen
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Many tests performed in Coagulation laboratories have a direct impact on patient management and inaccurate results in relation to familial and acquired bleeding and thrombotic disorders could have very serious consequences for patients. Accurate and reliable results are needed when testing is performed in relation to possible familial haemostatic disorders where laboratory error may lead to misdiagnosis. The pre-analytical, analytical and post-analytical phases of testing are all of great importance. Assessment of analytical quality should include participation in independent external quality assessment/proficiency testing programmes. For a laboratory to reach the quality required for accreditation to ISO standards it is a requirement that the laboratory participates in an inter-laboratory comparison programme appropriate to the examination and interpretation of such examination results (ISO 15189 2012). The ISO 15189 standard requires that the inter-laboratory comparison programme chosen by the laboratory should provide clinically relevant challenges that mimic patient samples and have the effect of checking the entire examination process, including pre-examination and post-examination procedures, where possible. EQA programmes have focussed on the analytical component but studies and articles always show that more errors occur in the pre-analytic phase than any other.

EQA programmes are encouraged by ISO to address the pre-analytic phase. This can be done in a number of ways and in future this will increasingly be included in EQA repertoires (ISO 17043). One approach is to use questionnaires about practice with feedback on how an individual centre compares to other centres. Another option could be use of EQA materials that contain interfering substance such as haemoglobin. The third option is to record error rates supplied by participants which can be compared to quality standards. All of these will likely increase in future EQA programmes.

Perhaps the most important component of laboratory testing is the post-analytical interpretation since it is the way that results are interpreted that impacts most on patient management. An example of post analytical assessment in EQA programmes is the use of D-dimer to exclude possible VTE. In the UK NEQAS Pint of Care D-dimer programme participants are invited to perform a D-dimer test on an EQA sample and use their local result along with a clinical scenario and pre test probability score such as the Wells score to decide whether VTE can be excluded or not. Their interpretation is then assessed. In future EQA programmes will have to address the post analytical phase more in this way.

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SPECIAL PLENARY 1: EVOLVING REGULATION OF LABORATORY DEVELOPED TESTS

(5) FDA’S INITIATIVE TO REGULATE LAB-DEVELOPED TESTS (LDT) WILL HARM PATIENTS AND ACADEMIC PATHOLOGY

Edward R. Ashwood, MD
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In October 2014, the FDA issued two draft guidance documents providing a risk-based framework for the regulation of Lab Developed Tests (LDTs). The proposals would place LDT regulation under the agency’s oversight authority based on the Medical Device Amendments of 1976. By issuing these draft two guidances, the FDA acts as if Congress granted it this expansive authority almost 40 years ago. However, the 1976 Act does not mention laboratories or laboratory testing services. Also, the Act specifically denies FDA the authority to regulate the practice of medicine. Thus, the FDA proposes to oversee LDTs as if these services were in vitro diagnostic devices, a sweeping shift from the agency’s 38-year history of exercising “enforcement discretion” for LDT. FDA lacks the statutory authority to regulate laboratory-developed testing services. While Congress conferred upon FDA the authority to regulate medical devices, they conferred oversight of clinical laboratories to Centers for Medicare and Medicaid Services (“CMS”) through the comprehensive Clinical Laboratory Improvement Amendments of 1988 (“CLIA”). This law was specifically designed for clinical laboratories and their tests. The proposed changes will increase costs for laboratories and healthcare. While it is possible that increased regulation may increase patient safety, there is little evidence that LDTs have been a significant source of safety issues. Although substandard tests are sometimes marketed, these events are rare and are quickly corrected. The costs of complying with FDA regulation are real. For example, ARUP offers approximately 584 Class II tests and 61 Class III tests on our current test menu. The cost of pre-market submission for class 2 is $50,000-$250,000 per test, and for class 3 is $2,500,000-$5,000,000 per test. The total cost would be about $316 million. Many of these tests are low volume and do not provide sufficient revenue to justify the approval cost. We would abandon about 410 tests. In addition, the financial constraints resulting from approval costs will stifle the improvement of older tests. More than half of ARUP’s R&D resource is used currently for test improvement. If the guidances are enforced, that improvement effort will be curtailed. Healthcare has benefited from innovative efforts within laboratories to advance and update testing methods in a timely and medically relevant way. The need for submissions and review will seriously reduce the innovation that drives laboratory medicine. The potential benefits of increased regulation are speculative. It is not clear that FDA regulation of diagnostic testing would lead to significant increases in patient safety. On the other hand, the impacts of regulation are very clear: less innovation, increased costs, reduced competition, and fewer choices for doctors and patients.

References

2. FDA Notification and Medical Device Reporting for Laboratory Developed Tests (LDTs); Draft Guidance, Docket number: FDA-2011-D-0357. 79 FR 59779 (Oct 3, 2014)
(7) CHALLENGES IN LABORATORY MEASUREMENT OF NEXT GENERATION RECOMBINANT FVIII AND FIX HEMOPHILIA REPLACEMENT PRODUCTS

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A number of recombinant factor VIII and IX replacement products are currently in late stage development or have recently been approved in both US and/or Europe. One-stage factor assays based on the activated partial thromboplastin time (APTT) are the most commonly used assays for measuring FVIII and FIX activities in clinical laboratories. A wide variety of instruments and APTT reagents for factor testing are currently in use. In addition, reagent dependent responses for some of the new factor replacement products in factor assays have been described. As a result, laboratory monitoring of these new products post-market approval has been subject of intense discussion among manufacturers, regulators and the clinical community. This presentation will review available data for novel recombinant factor VIII and IX replacement products in commonly used APTT reagent systems, as well as select chromogenic assays and summarize approaches currently under consideration by product manufacturers to ensure adequate measurement of these products by clinical laboratories.

References


(8) THE NIJMEGEN MODIFIED BETHESDA ASSAY: NEW CONCEPTS

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Development of neutralizing antibodies to replacement products during treatment of Hemophilia A and B patients is a serious adverse event and regular laboratory monitoring for their occurrence is required. The Nijmegen modification of the classic Bethesda assay was introduced in 1995 to improve sensitivity and specificity of inhibitor detection and is the factor inhibitor assay recommended by the International Society for Thrombosis and Haemostasis (ISTH). Since its introduction, however, external quality assessment programs demonstrate high inter-laboratory variability with coefficients of variation (CVs) often greater than 30%. This in part is due to the many steps and complexities of the assay and the lack of published data as to how minor variations in procedure may impact results. Several aspects of the Nijmegen-modified Bethesda assay procedure that potentially contribute to the observed inter-laboratory variability will be discussed.

References

(9) PEARLS AND PITFALLS IN FACTOR INHIBITOR AS-
SAYS
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The proper performance and interpretation of factor inhibitor assays is a critical role for the hemostasis laboratory. Both false positive and false negative inhibitor assays may be reported, leading to serious patient mismanagement. Knowledge and recognition of common causes of both false positive and negative results can aid in the identification of these potential pitfalls and will be reviewed. Safeguards to reporting accurate factor inhibitor assays will be discussed and include: initial characterization of the sample, using the Nijmegen modification, properly performing and interpreting an incubated mixing test in conjunction, and performing two dilutions for each dependent dilution in the factor inhibitor assay.

References

CONCURRENT 2: CELLULAR CHARACTERIZATION: FROM CYTOGENETICS TO FLOW CYTOMETRY
(10) DO YOUR FLOW CYTOMETRIC LDTS CONFORM TO THE ICSH-ICCS VALIDATION GUIDELINES?
Fiona E. Craig, MD
University of Pittsburgh School of Medicine

Take ownership of developing, validating and maintaining the quality of clinical assays run in your flow cytometric laboratory. The ICSH-ICCS practice guidelines describe how to take clinical laboratory practices developed for soluble analytes and apply them to cell-based fluorescence assays, even in the absence of available reference preparations. This presentation will describe how to optimize instrument and reagent performance, and then perform a validation study prior to implementing a flow cytometric assay for leukemia and lymphoma immunophenotyping. A validation plan will be outlined that addresses clinical sensitivity and specificity, analytical sensitivity and specificity, precision and stability, using examples of chronic lymphocytic leukemia (CLL). The presentation will conclude by addressing the additional steps required to demonstrate the performance of quantitative or semi-quantitative assays for CLL minimal residual disease and monoclonal B lymphocytosis detection, such as determining the lower-limit of quantitation.

References
FLOW CYTOMETRY OF LEUKOCYTES IN NON MALIGNANT HEMATOLOGICAL DISORDERS
Marie Christine Bene
CHU Nantes, France

Multiparameter flow cytometry (MFC) is widely used in hematology laboratories for the diagnosis and follow-up of leukemias, lymphomas and, increasingly, myelodysplasia. This technology can also be very useful in non-malignant or pre-malignant disorders through the analysis of peripheral blood samples. The exploration of leukocyte subsets can provide clinically useful clues for the management of patients in several context. Abnormal lymphocyte subsets can be identified in the context of hypereosinophilia. Infection may lead to an increase of non-classical monocytes. Persistent neutropenia may be associated to an increase in large granular lymphocytes which are worth immunophenotyping for deeper characterization. Paroxysmal nocturnal hemoglobinuria also now benefits from an immunophenotypic diagnosis and follow up. Some congenital disorders can also be identified or ruled out by rapid MFC techniques, for instance congenital spherocytosis or Glanzmann thrombasthenia.

FLOW CYTOMETRY IMMUNOPHENOTYPING IN INTEGRATED DIAGNOSTICS OF PATIENTS WITH NEWLY DIAGNOSED CYTOPENIA
Anna Porwit, MD, PhD
Department of Pathobiology and Laboratory Medicine, University of Toronto

Acute leukemia, myelodysplastic syndromes (MDS), myeloproliferative neoplasms and lymphomas are the most prevalent diagnoses in adults presenting with new onset cytopenia. In patients with blasts <10% and no clear involvement by hematological malignancy based on cytomorphological evaluation of bone marrow (BM) smear, we apply the one-tube 10-color 14-antibody flow cytometry screening panel (CD4+Kappa/FITC, CD8+Lambda/PE, CD3+CD14/ECD, CD33 PC5.5, CD20+CD56/PC7, CD34 APC, CD19 APC/AlexaF700, CD10 APC Alexa F750, CD5 Pac. Blue, CD45 KO). The screening panel has been developed to detect major B- and T-cell abnormalities, to evaluate numbers of cells in the blast region (CD45 dim), and to give insight into myeloid BM compartment, including calculation of four-parameter score for MDS-related abnormalities (so called Ogata score). In cytopenic patients who present with >10% <20% blasts in blood or BM smears, a comprehensive three-tube 10-color panel of surface markers is used up-front. The analysis is focused on the detection of abnormal antigen expression patterns in blast, granulopoietic, monocyte and erythroid compartments, by comparison to patterns seen in normal/reactive BM. Patterns characteristic for MDS are defined according to the guidelines developed by International/European LeukemiaNet Working Group for Flow Cytometry in MDS. In patients with >20% blasts, additional tube is added to allow detection of cytoplasmic/nuclear markers necessary to diagnose mixed phenotype acute leukemia.

References
(13) DEMOCRATIZING MOLECULAR DIAGNOSTICS FOR THE DEVELOPING WORLD
Benjamin Pinsky, MD, PhD
Stanford University School of Medicine

Infectious diseases account for significant morbidity and mortality worldwide and disproportionately impact populations with limited resources. While nucleic acid-based testing has revolutionized infectious disease diagnosis and monitoring in resource-rich settings, it is only recently that technological developments have allowed molecular testing to be carried out where resources are limited and the infrastructure for conventional molecular testing is nonexistent. In this presentation, near-care and point-of-care, sample-to-answer molecular diagnostics for infectious diseases will be covered, including tests for HIV-1, Mycobacterium tuberculosis, and Ebola virus Zaire (1-5).

References

(14) CLINICAL UTILITY OF GENOME AND EXOME SEQUENCING
Jason Merker, MD, PhD
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Health care providers are more frequently using genome and exome sequencing in the evaluation of patients with unexplained heritable disease. Genome and exome sequencing examine approximately 20,000 genes. Consequently, these methods have the potential to identify genetic variants that cause a variety of heritable diseases. This potential must be balanced against numerous technical, informatics, interpretive, ethical, and other challenges associated with the size and complexity of the generated data. I will describe our experience establishing a clinical genomics service at an academic medical center. This will include discussion of workflow, advantages and limitations of such testing, and case examples. I will then discuss our initial efforts to use genome sequencing to identify the molecular etiology in adult and pediatric patients with unexplained genetic disease.

References
Richards S et al. Standards and Guidelines for the Interpretation of Sequence Variants: A Joint Consensus Recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015 [Epub ahead of print]
Myeloid malignancies including acute myeloid leukemia (AML) and myelodysplastic syndromes (MDS) are a heterogeneous group of disorders that share a common biology and are a major source of morbidity and mortality. In the last several years studies using next generation sequencing (NGS) have identified a core set of recurrently mutated myeloid malignancy genes in the majority of AML and MDS patients, including those with normal cytogenetics. DNA-level mutations in several of these genes including NPM1, FLT3, and CEBPA-AML and ASXL1, ETV6, EZH2, RUNX1, and TP53 in MDS are associated with changes in patient outcomes and are now tested for in clinical laboratories. In addition to providing prognostic information, these gene mutations can be used to monitor patient disease burden through the use of ultra-sensitive detection techniques. In this review we will focus on the clinical utility of various NGS based methods including whole genome sequencing, exome sequencing, and targeted panel based sequencing in the initial diagnosis and management of AML and MDS and cover recent methodological advances for the molecular monitoring of AML and MDS.

References


CONCURRENT 4: IRON DISORDERS AND NUTRITIONAL ANEMIAS

(16) ANALYSIS OF IRON STATUS IN HEMATOLOGIC DISORDERS

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Diseases associated with iron deficiency and iron overload affect a large populations in both the developed and developing worlds. Maintaining iron balance is critical for hemoglobin synthesis. No physiologic mechanism for iron excretion exists in humans such that iron overload, either as a primary disorder of inappropriately high iron absorption, due to frequent red blood cell transfusions, or resulting from ineffective erythropoiesis, if untreated, leads to significant morbidity and mortality. Thus, both phlebotomy and iron chelation are used to manage different iron overloaded patients.

Data suggest that markers of iron overload portend a relatively poor prognosis and iron chelation therapy is associated with prolonged survival in transfusion-dependent patients. On the opposite end of the iron spectrum, although treating primary iron deficiency anemia is relatively straightforward, the complexity of iron delivery for erythropoiesis in inflammatory states leaves patients insensitive to erythropoietin stimulating agents. A controversy in what constitutes indication for iron supplementation in patients with renal failure suffers from insufficient data that iron supplementation, even when no systemic iron deficit is identified, causes harm. Recently renewed interest in the iron field was heralded by the discovery of hepcidin, a serum peptide hormone and the main negative regulator of body iron. Evidence from β-thalassemia suggests that regulation of hepcidin by erythropoiesis dominates regulation by iron and multiple pathologic and physiologic erythroid regulators of hepcidin have recently been put forth. This finding has stimulated a new wave of exploration into the crosstalk between iron metabolism and erythropoiesis and galvanized the translation of novel diagnostic parameters and therapeutic agents for human use. The purpose of this presentation is to characterize and discuss the novel potential and theoretical tools available to better define iron status in diseases such as hereditary hemochromatosis, β-thalassemia, myelodysplastic syndrome, and renal failure ultimately providing a more nuanced, individual, evidence-based approach to therapeutic indications. We anticipate that additional novel tools for measuring iron status and delineated molecular pathways in various disease states will provide a deeper understanding of how iron metabolism and erythropoiesis co-regulate and improve clinical management of these patient populations.

References

Anemia affects approximately one quarter of the world’s population and is associated with an increased risk of adverse pregnancy and birth outcomes, impaired mental and motor development in infants and children, and reduced economic capacity and physical performance in adults. Anemia can have many causes, including iron and other nutritional deficiencies, infection, inflammation and disorders which affect hemoglobin or red blood cells. After iron deficiency, which accounts for approximately 50% of the global anemia burden, the most common causes are hookworm, sickle cell disorders, thalassemias and malaria. The purpose of this paper is to provide an overview of the public health interventions aimed at addressing anemia, including the related evidence base and global guidance, and outline considerations to increase the impact of these interventions in program settings. Public health interventions to control anemia can be broadly divided into dietary modification, supplementation, fortification, infectious disease control and clinical practices. Dietary modification often focuses on increasing intake of foods rich in bioavailable iron, especially meat, but can also include strategies to increase iron bioavailability. Oral iron supplementation is a common approach to prevent or treat iron deficiency anemia in vulnerable groups; a large body of evidence shows that supplementation can greatly reduce anemia. Point-of-use fortification with multiple micronutrient powders (MNP), which are vitamins and minerals in powder form to be added to food shortly before consumption, is increasingly being used as an alternative to supplementation (with similar impact) for children and can be provided in the home or other settings such as schools. Commercial food fortification is used in many countries to add iron and other micronutrients during processing to widely consumed foods as part of an anemia prevention strategy, though limited research is available on its impact. Biofortification is another more recently developed type of fortification which involves breeding of staple crops improve their nutritional value; it has not been widely adopted though the evidence base is developing. Certain strategies to control parasitic infection which can cause anemia, such as malaria or hookworm may also help address anemia. Finally, delayed clamping of the umbilical cord following birth is a clinical practice which has been demonstrated to increase newborn hemoglobin levels. The World Health Organization (WHO) has issued guidelines or recommendations for many of the above-mentioned interventions as options for countries to consider based on the local anemia burden, some of which include specific vulnerable populations such as those living with HIV or tuberculosis. Following a new evidence review and guideline development process, in the past few years guidelines were issued on iron and folic acid supplementation in menstruating, pregnant and postpartum women, MNP for children 6-23 mo of age, and delayed cord clamping. Some WHO and other global guidelines relating to healthcare or feeding, especially for pregnant women and young children, include broad recommendations to promote consumption of iron-rich foods. WHO recommendations also exist for fortification of wheat or maize flour, with iron and other nutrients depending on the nutrition profile of the population. Due to concerns of potential for iron interventions to increase illness in malaria-affected areas, all new WHO guidelines recommend that iron supplements or MNP are provided in conjunction with efforts to prevent, diagnose and treat malaria. WHO recommendations also exist for malaria control strategies and for periodic preventive chemotherapy (deworming) for groups at risk of hookworm and other helminth infections that can cause anemia. Most of the current recommendations mentioned above are summarized in the WHO Essential Nutrition Actions report (see reference list). Global anemia prevalence has remained largely unchanged in the past 2 decades despite the availability of solutions and so many countries having anemia control policies, and to a certain extent programs, in place. This may be due to a variety of context-specific factors though we argue that there are key elements necessary for success and impact, relating to an enabling environment, policy and program design, intervention delivery, behaviour change strategies, and monitoring, surveillance and evaluation. Ensuring these key elements are considered and well-implemented will help maximize the impact of anemia control programs and improve the lives of millions of people.

References


(18) DIAGNOSTIC TESTING FOR HEMOCHROMATOSIS AND IRON OVERLOAD
Paul C. Adams, MD
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Hemochromatosis is the most common genetic disease in Northern European populations. Body iron stores progressively increase in most patients which can lead to cirrhosis of the liver, hepato cellular carcinoma, heart failure, arthritis, and pigmentation. Simple blood tests such as the serum ferritin and transferrin saturation are useful to suggest the diagnosis which can be confirmed in most cases with a simple genetic test for the C282Y mutation of the HFE gene. However these blood tests are often misinterpreted and there are rare patients with iron overload without HFE mutations. A diagnostic approach is presented based on a large referral practice and a population based study (HEIRS) which screened for iron overload in 101,168 participants.

References

ORAL ABSTRACT SESSION: CELLULAR ANALYSIS & MORPHOLOGY

(19) REPORT ON HEMATOFLOW™ FLAGGING SYSTEM IN A ROUTINE HEMATOLOGY LABORATORY
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Introduction: The complete blood cell count and white blood cell differential are the first step in the biological diagnosis of hematological diseases. In case of flag alarm, the automated differential cannot be validated and the operator must activate a blood smear review. Unfortunately, microscopic examination is still today the reference method despite its lack of sensitivity and reproducibility. The HematoFlow™ (Beckman Coulter) system is the first alternative flow cytometry commercialized method for the routine differential. Methods: We previously described in 2013 (poster ISLH Toronto) in a retrospective study of 6462 cases, a new HematoFlow™ Flagg system (HFS) allowing a best detection of sample abnormalities even for rare events. After 2 year work, a provisional patent application (87904-896088-028000US) followed and definitive results are under publication in Cytometry. Here are reported a summary of this results and of an additional prospective series of 15335 cases. Results: In the retrospective series, from an initial workload of 32231 cases analyzed by an automated instrument (LH 785 Beckman Coulter), 6462 cases (20.2%) were analyzed using HFS. After application of HematoFlow™ autovalidation process, 3496 (54.1%) samples could be autervalidated without any false negative case. The remaining 45.9% of samples required visual validation of histograms. Among them, only 812 (12.6%) required microscopic examination, leading to an economy of 5650 (87.4%) slide examinations. Finally, from 32231 cases, only 3.1% remained necessary after running HematoFlow™. A special attention was brought to validation of blast cells and immature granulocytes (IG) percentages (%) and flag alarm provided by the HematoFlow™ system. Blast cell % and IG% were compared to morphologic results when no HFS flag was present. Considering morphologic examination as the reference method, despite its lack of sensitivity and reproducibility. Review. Unfortunately, microscopic examination is still today the reference method despite its lack of sensitivity and reproducibility. The HematoFlow™ (Beckman Coulter) system is the first alternative flow cytometry commercialized method for the routine differential. Conclusion: We previously described in 2013 (poster ISLH Toronto) in a retrospective study of 6462 cases, a new HematoFlow™ Flagg system (HFS) allowing a best detection of sample abnormalities even for rare events. After 2 year work, a provisional patent application (87904-896088-028000US) followed and definitive results are under publication in Cytometry. Here are reported a summary of this results and of an additional prospective series of 15335 cases. Results: In the retrospective series, from an initial workload of 32231 cases analyzed by an automated instrument (LH 785 Beckman Coulter), 6462 cases (20.2%) were analyzed using HFS. After application of HematoFlow™ autovalidation process, 3496 (54.1%) samples could be autervalidated without any false negative case. The remaining 45.9% of samples required visual validation of histograms. Among them, only 812 (12.6%) required microscopic examination, leading to an economy of 5650 (87.4%) slide examinations. Finally, from 32231 cases, only 3.1% remained necessary after running HematoFlow™. A special attention was brought to validation of blast cells and immature granulocytes (IG) percentages (%) and flag alarm provided by the HematoFlow™ system. Blast cell % and IG% were compared to morphologic results when no HFS flag was present. Considering morphologic examination as the reference method, despite its lack of sensitivity and reproducibility. Review. Unfortunately, microscopic examination is still today the reference method despite its lack of sensitivity and reproducibility. The HematoFlow™ (Beckman Coulter) system is the first alternative flow cytometry commercialized method for the routine differential. Conclusion: We previously described in 2013 (poster ISLH Toronto) in a retrospective study of 6462 cases, a new HematoFlow™ Flagg system (HFS) allowing a best detection of sample abnormalities even for rare events. After 2 year work, a provisional patent application (87904-896088-028000US) followed and definitive results are under publication in Cytometry. Here are reported a summary of this results and of an additional prospective series of 15335 cases.

Implementation of the HFG flagging system in this study should improve productivity in the haematology laboratory and diagnosis quality. The precise quantification of normal and abnormal cells is important in the follow-up of hematological diseases and could be more rapidly implemented for a better management of patients.
(20) PLATELET AND MONOCYTE ACTIVATION IN HCV-INDUCED CHRONIC LIVER DISEASE
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Introduction: Chronic liver disease is characterized by an inflammatory and fibrotic process with a progression from chronic hepatitis to cirrhosis. Monocytes play a crucial role in the pathogenesis of inflammation and fibrosis in chronic liver diseases together with blood platelets. We aimed in this study to assess platelet-monocyte aggregates in the peripheral blood of patients with HCV induced chronic liver disease and their relation to the degree of hepatic insufficiency and hemostatic imbalance as well as their relevance to aggravating hematemesis in these patients. Methods: 60 patients with HCV induced chronic liver disease, classified according to Child Pugh into 4 groups each with 15 patients (A, B, C and C during acute attack of hematemesis) were included together with 15 healthy control subjects. Flow-cytometry Immuno-phenotype characterization of platelet-monocyte aggregates (CD41/CD45) on activated blood monocytes (CD11b and CD14) and activated blood platelet populations (CD61 and CD62P) in different groups was carried out. The circulating blood levels of monocyte activation marker (MCP-1) and platelet activation markers (PF4 and sP-selectin) were also determined in different groups using immunological assays. Results: There was a stepwise increase of monocytes and platelets in parallel with the severity of liver disease. A marked decrease in platelet count concomitant with an increase in the surface expression of CD61 on platelets was also noticed in different patient groups compared to healthy subjects. A significant increase in the surface expression of each of CD11b, CD14 and CD41/45 on monocytes was observed in different patients’ groups, especially cases with acute attacks of hematemesis. There was a significant progressive increase in the surface expression of CD62P and CD41/CD45 on peripheral blood platelets and in the circulating levels of sP-selectin and PF4, indicating increased activation of peripheral blood platelets, in different groups of patients especially cases with acute attacks of hematemesis. Conclusions: The progressive increase in monocytes and platelets activation with the simultaneous formation of monocyte-platelet aggregates in our patients may contribute to progressive liver injury. Monocytes play a crucial role in the pathogenesis of liver damage and liver cirrhosis, and as platelets, participate in pathogenesis of chronic liver diseases. We, therefore, may suggest that the blockage of platelet and monocyte activation may diminish thrombocytopenia and liver cirrhosis intensification in patients with chronic hepatitis C virus.

(21) INCREASED BONE MARROW DENSITIES OF MAST CELLS, TUMOR-ASSOCIATED MACROPHAGES AND ANGIogenesis IN PLASMA CELL MYELOMA: AN IMMUNOMORPHOMETRIC ANALYSIS
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Introduction: Multiple myeloma is characterized by wide biological and clinical heterogeneity, that is attributable both to malignant plasma cells as well as their interactions with the host microenvironment. Unlike the extensively studied extracellular matrix, humoral components, angiogenesis and lymphocyte subsets, much less literature exists about tumor-associated macrophages (TAMs) and mast cells in myeloma. In recent years, both cell types have been shown in other settings to augment pathologic angiogenesis, a phenomenon vital to myeloma progression. Mast cell densities are increased in myeloproliferative neoplasms and many solid tumors. TAMs are now known to be of prognostic value in Hodgkin and non-Hodgkin lymphomas (NHLs). Such information is however, relatively scanty in myeloma. Methods: Pre-treatment bone marrow (BM) biopsies from 41 myeloma patients diagnosed as per the 2008 WHO criteria were stained immunohistochemically for CD34 (Clone QBend10, Cat. No. IS63230), CD68 (Clone PG-M1, Cat. No. IS61330) and mast cell tryptase (Clone AA1, Cat. No. M705229) antibodies using the EnVision FLEX Mini Kit (High pH; Link, Cat. No. K802321) from Dako, Glostrup, Denmark. Twenty staging BM biopsies from patients with NHL who had normal hematologic parameters were also similarly stained as controls. Morphometric analyses for microvessel density (MVD), microvessel surface area (MSA), TAMs and mast cell density were done using the Image J™ software (http://imagej.nih.gov/ij/) and the Image ProPlus 4.5 software (Media Cybernetics, Maryland, USA). All analyses were performed blinded to the patients’ clinical backgrounds or other laboratory data. Results: CD68-positive macrophages, tryptase-positive mast cells, MVD as well as MSA were all significantly increased in patients as compared to controls. TAMs tended to be dispersed in both the plasma cell-rich marrow spaces as well as in spaces with residual hematopoietic elements. In contrast, mast cells tended to cluster in the peritumoral regions with the highest MVD. Morphometrically too, the mast cell density showed a highly significant positive correlation with MVD and MSA. TAMs appeared independent of MVD, however, correlated significantly with plasma cell percentage, hemoglobin and lactate dehydrogenase. Legend to figure 1: [A] shows prominent angiogenesis, [B] high mast cell density, and [C] increased TAMs in patients with myeloma (400x magnification, immunoperoxidase staining, hematoxylin counterstain). Conclusions: Immunohistochemical staining on the BM biopsy followed by image analysis represents an objective and convenient method to quantitatively...
assess microenvironmental components in myeloma. Mast cell density’s correlation with MVD and MSA supports a major role for these cells in myeloma angiogenesis. CD68 requires further study as a potential indicator of disease prognosis in these patients.

(22) PERFORMANCE AND LIMITATIONS OF AUTOMATED CELL COUNTING, AUTOMATED MORPHOLOGICAL ANALYSIS, AND MANUAL MICROSCOPY IN THE EVALUATION OF LEUKOCYTE DIFFERENTIAL COUNTS DURING A DENGUE FEVER OUTBREAK

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University of Campinas - UNICAMP Campinas, Brazil

Introduction: Complete blood counts are often requested by clinicians for patients with febrile diseases. During a dengue fever outbreak, our laboratory received an increased number of samples whose manual white blood cell (WBC) differential count was required due to flags for atypical lymphocytes (AL) or immature granulocytes (IG), along with frequent leukopenia, which significantly slows the conclusion of the analyses. During an outbreak, the use of automated morphological analysis systems, such as Cellavision™ DM96, may prove to be useful by enabling a quick morphological pre-classification of leukocytes. We aimed to evaluate the use of this platform and compare with data from automated cell counting (percentage of IGs and pseudobasophilia, previously reported to correspond to ALs), and from concurrent manual microscopy.

Methods: A total of 190 blood samples with clinical suspicion of dengue fever were analyzed with a Sysmex XE-5000 equipment at the University Hospital of the University of Campinas, Brazil. WBC differential counts were performed by two experienced laboratory personnel by manual microscopy and using CellaVision™ DM96 using the same blood smears. Statistical analysis was performed using GraphPad Prism 6.0 software.

Results: Eighty-three (43%) samples had a WBC count under 4,000/µL (range 1,400-15,520). Automated cell count detected 11 samples with IG over 1% (range 1.1-3.6%), while 54 samples under manual microscopy and 75 under automated morphological analysis showed over 7% band cells (range 8-46%) or over 1% of more immature granulocytes. Pseudobasophilia (over 1% of basophils as determined by the XE-5000) was present in 68 samples (range 1.1-9.1%) and correlated with the percentage of ALs counted with manual or automated microscopy (Spearman r²=0.64 and 0.60, respectively, P < 0.001). Both microscopy modalities correlated in the percentage of AL and band cells (P < 0.001), although the strength of these correlations remained moderate. Conclusions: Pseudobasophilia corresponded to atypical lymphocytosis confirmed by both manual and automated microscopy. Automated IG count failed to detect WBC left shift in up to 33% of our samples, possibly due to the predominance of band cells in dengue cases. Differences in morphological analysis between manual and CellaVision™DM96-assisted microscopy may represent the result of non-uniform distribution of the blood cells on the slide. Automated morphological analysis systems may be nonetheless be useful and sufficiently reliable when the assessment of atypical lymphocytosis and left shift in leukopenic samples is frequently required, such as in the setting of an outbreak of dengue fever.
ORAL ABSTRACT SESSION: COAGULATION & PLATELETS

(23) MULTIPLEX PLATELET ACTIVATION TEST TO MEASURE DIFFERENTIAL PLATELET ACTIVATION.
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Introduction: P2Y12 inhibitors are prescribed to prevent secondary cardiovascular events. Although successful on population level, there are still patients on P2Y12 inhibitors who either develop thrombosis or bleeding complications. This complication rate may be reduced by tailored adjustment of treatment intensity, based on platelet function monitoring. Several global haemostasis tests have been used for these purposes, but failed by lack of sensitivity or specificity. The aim of the current study was to investigate the impact of P2Y12 inhibitors on different platelet activation responses, induced by multiple agonists. Methods: We have developed, optimized and evaluated a novel sensitive multiplex Platelet Activation Test (PACT) to assess on-treatment platelet reactivity, based on quantification of alpha granule release and activation of the αⅡbβ3 receptor. The PACT monitors different platelet activation pathways. Results: Using the PACT, we showed that ADP mediated platelet activation can be completely blocked with high P2Y12 inhibitors, while PAR1, PAR4 and GPVI induced fibrinogen-binding capacity is strongly inhibited. In contrast to fibrinogen-binding capacity, PAR1, PAR4 and GPVI induced P-selectin expression is only minimally affected by P2Y12 inhibitors, indicating a differential activation pattern. All findings are confirmed in vitro, under flow and in blood of patients on clopidogrel treatment. Conclusions: Conclusion: Secondary platelet activation via the P2Y12 receptor enhances fibrinogen binding to activated platelets upon PAR-1, PAR-4 and GPVI induced activation while it has minor impact on P-selectin expression. These findings indicate that P2Y12 inhibitors impair platelet thrombus formation, without affecting granule release. Our study provides new tools to monitor on-treatment platelet function in individual patients, which may be crucial for adjustment of treatment intensity with P2Y12 inhibitors.

(24) A HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY METHOD FOR THE SEROTONIN RELEASE ASSAY IS EQUIVALENT TO THE RADIOACTIVE METHOD
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Introduction: Laboratory tests for heparin-induced thrombocytopenia (HIT) include immunoassays and functional platelet activation assays, such as the serotonin release assay (SRA). The SRA is considered the gold-standard test, with >90% sensitivity and specificity for HIT. The historic SRA method uses platelets loaded with radiolabeled serotonin to evaluate platelet activation by HIT immune complexes. However, a non-radioactive method is desirable to avoid the complexities and potential hazards of working with radioactivity. We report on the robust performance characteristics of a high-performance liquid chromatography (HPLC) SRA method currently in use in our coagulation reference laboratory. Methods: We validated the performance characteristics of an HPLC SRA method. Assay reactions included 75 µL washed platelets, 20 µL heat-inactivated serum, and 5 µL unfractionated heparin (0.1 units/mL or 100 units/mL). Released serotonin was quantified by HPLC on an Agilent 1260 instrument using fluorescent detection. Results were expressed as % release and classified as positive, negative, or indeterminate based on previously published cutoffs. Concordant classifications were observed in 230 samples (22 positive, 207 negative, 1 indeterminate), for a concordance rate of 92%. The 20 discordant samples included various combinations of results with no trend toward any particular discordant pattern. Between-run precision studies were performed on the HPLC SRA using pools of positive, weak positive, negative, and indeterminate-reacting sera tested in replicates of 4 over 5 days. Standard deviation was used as an estimate of imprecision and was < 6 (% release) for the positive, weak positive, and negative pools and < 13 (% release) for the indeterminate pool. Frozen sera from 41 healthy individuals with no history of heparin use or HIT tested negative in the HPLC SRA. Stability studies demonstrated stability of HPLC SRA results after sera were subjected to 2 freeze-thaw cycles or up to a week of refrigeration. Conclusions: The HPLC SRA has robust performance characteristics, equivalent to the historic radioactive method, but avoids the complexities of working with radioactivity.
CLINICAL UTILITY OF ADAMTS13 ASSAY IN DIAGNOSIS OF THROMBOTIC THROMBOCYTOPENIC PURPURA

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Introduction: Thrombotic thrombocytopenic purpura (TTP) is a rare life-threatening disease and rapid diagnosis and emergent plasma exchange (TPE) can improve survival. The “classic pentad” includes microangiopathic hemolytic anemia (MAHA), thrombocytopenia, renal dysfunction, neurologic abnormalities, and fever; however, these are rarely all present. Given this clinical variability, assays to detect ADAMTS13 activity and inhibitor are becoming crucial for early TTP diagnosis. In this study we analyze ADAMTS13 activity and inhibitor levels in patients being evaluated for TTP to determine the clinical utility of these assays for TTP diagnosis. Methods: All ADAMTS13 activity and inhibitor levels performed at our institution to evaluate for TTP over an 18 month period were reviewed. ADAMTS13 activity was measured using a fluorescence resonance energy transfer assay (American Diagnostica Inc/Sekisui, Stamford, CT), and ADAMTS13 inhibitor level (Bethesda Unit) was calculated after 1:1 mixing of patient and normal plasma and measurement of residual activity. Patient age, gender, symptoms, and relevant laboratory values were obtained from the electronic medical record. Sensitivity and specificity for different cut-offs of ADAMTS13 activity, and clinico-laboratory findings in these patients were reviewed. Results: ADAMTS13 assays were performed on 79 patients of whom 25 were clinically diagnosed with TTP. Patients with TTP had an average ADAMTS13 activity of 15.4% (median 5%, range 0-66%) versus 65.2% in non-TTP patients (median 62%, range 10-131%; p<0.0001). Clinico-laboratory findings which were significantly different between TTP and non-TTP patients are listed in the table below. Sensitivity and specificity for ADAMTS13 activity cut-offs are shown in figure 1; <30% had the best overall performance using an ROC analysis (AUC of 0.921, p<0.0001). 4 TTP patients had mildly decreased activity (30-67%; average 57.8%); inhibitor assays were positive in 1/4.

<table>
<thead>
<tr>
<th>Clinico-laboratory parameters</th>
<th>TTP (n=25)</th>
<th>Non-TTP (n=54)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thrombocytopenia (mean platelet count k/ul)</td>
<td>24 (55.1)</td>
<td>38 (117.9)</td>
<td>0.015 (0.0005)</td>
</tr>
<tr>
<td>MAHA</td>
<td>22</td>
<td>20</td>
<td>0.0001</td>
</tr>
<tr>
<td>Neurologic symptoms</td>
<td>14</td>
<td>12</td>
<td>0.005</td>
</tr>
<tr>
<td>Schistocytes a6/fp</td>
<td>14</td>
<td>8</td>
<td>0.0003</td>
</tr>
<tr>
<td>ADAMTS13 inhibitor &gt;0.4</td>
<td>18</td>
<td>12</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

Conclusions: Our data demonstrate that ADAMTS13 activity of less than 30% shows high sensitivity and specificity for diagnosing TTP. Assays for ADAMTS13 function inhibitor and autoantibody may be added in an algorithmic manner to maximize the diagnostic utility of this assay. ADAMTS13 activity should be evaluated in the clinical context of each patient, and in patients with a high pre-test probability of TTP, a higher cutoff point could be used.

ASSESSMENT OF THE DIAGNOSTIC VALUE OF PLASMA LEVELS, ACTIVITIES AND THEIR RATIOS OF VON WILLEBRAND FACTOR AND ADAMTS13 IN PATIENTS WITH CEREBRAL INFARCTION

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Introduction: Raised levels of von Willebrand Factor (VWF) and reduced levels of ADAMTS13 activity are associated with thrombosis. We aimed to investigate the relationships between plasma levels of VWF and ADAMTS13, their ratios, and the occurrence of cerebral infarction, and to understand the roles of VWF and ADAMTS13 in cerebral infarction. Methods: 94 cerebral infarction patients and 103 controls were analyzed. Plasma levels of VWF antigen (VWF:Ag), VWF:StFestocetin cofactor activity (VWF:Rcof), and VWF collagen binding activity (VWF:CB) were measured by ELISA, ADAMTS13 activity (ADAMTS13) was measured with FRETS-VWF73. The relationship between plasma levels and ratios of VWF and ADAMTS13 and the occurrence cerebral infarction were analyzed. Results: The levels of VWF:Ag and VWF:Rcof in cerebral infarction patients (median [IQR], 176% [114%-293%] and 197% [105%-381%]) were significantly higher compared with controls (105% [61%-145%] and 100% [60%-175%], p < 0.001). Levels of ADAMTS13 and ratios of VWF:CB/VWF:Ag, ADAMTS13/VWF:Ag and ADAMTS13/VWF:Rcof in patients (109% [80%-132%], 0.43 [0.28-0.90], 0.54 [0.32-0.99] and 0.57 [0.29-0.91], respectively) were significantly lower compared with controls (117% [103%-146%], 1.13 [0.72-1.81], 1.23 [0.79-2.16] and 1.18 [0.71-1.79], p < 0.01, respectively). However, there were no statistical differences between cerebral infarction and controls in VWF:CB (90% [53%-163%] vs 109% [73%-158%], P = 0.079), VWF:Rcof/VWF:Ag (0.95 [0.66-1.87] vs 1.18 [0.75-1.80], P = 0.405), or ADAMTS13/VWF:CB (1.05 [0.70-1.69] vs 1.10 [0.75-1.67], P = 0.694). The highest quartile of VWF:Ag (OR = 5.11, 95% CI, 1.49-17.50), VWF:Rcof (OR = 5.04, 95% CI, 1.62-15.66), and the lowest quartile of VWF:CB/VWF:Ag (OR = 5.91, 95% CI, 1.95-17.93), ADAMTS13/VWF:Ag (OR = 9.11, 95% CI, 2.49-33.33), ADAMTS13/VWF:Rcof (OR = 3.73, 95% CI, 1.39-10.03) have been found to have an association with cerebral infarction. Conclusions: Cerebral infarction patients displayed higher VWF:Ag and VWF:Rcof levels, and lower ADAMTS13, VWF:CB/VWF:Ag, ADAMTS13/VWF:Ag, ADAMTS13/VWF:Rcof levels compared to controls. An association was found between reduced levels of VWF:CB/VWF:Ag, ADAMTS13/VWF:Ag and ADAMTS13/VWF:Rcof ratios and cerebral infarction. Our data suggest that the joint analysis of VWF:Ag, VWF:Rcof, VWF:CB/VWF:Ag, ADAMTS13/VWF:Ag, ADAMTS13/VWF:Rcof are useful to predict the risk of cerebral infarction.
**ORAL ABSTRACT SESSION: FLOW CYTOMETRY & MOLECULAR ANALYSIS**

(27) ABSENCE OF CD24 EXPRESSION IN HAIRY CELL LEUKEMIA AND IDENTIFICATION OF A NORMAL CD24-NEGATIVE MEMORY B-CELL SUBSET: POTENTIAL CELL OF ORIGIN?

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**Introduction:** CD24 was originally defined as a B-cell associated antigen but is also expressed by granulocytes and epithelial cells. In normal B-cell differentiation, CD24 expression occurs early in bone marrow lineage commitment and is retained until the transition to plasma cell. CD24 expression density varies during B-cell differentiation with high density on B-cell precursors, intermediate density on transitional B-cells and low density expression on naïve B-cells. CD24 is down-regulated on follicle center B-cells and following egress from the follicle, is re-expressed at high density on most memory B-cells. **Methods:** Evaluation of routine clinical specimens between September 2003 and November 2014 included 47 cases of hairy cell leukemia and 7 cases of hairy cell leukemia variant for which CD24 expression had been assessed. CD24-positive cases were evaluated for both expression density and the percentage of positive cells. Samples were evaluated on an FC500 Beckman Coulter (BC) using BC antibodies (prior to September 2012) or on a Beckman Dickinson (BD) FACs Canto II using BD antibodies (October 2012 to present). **Results:** Of the HCL cases evaluated for CD24 expression, 15% of cases were positive (7/47). When present, CD24 showed partial, low-density expression ranging from 21-52% of cells with an average of 31% positive cells. Of the 7 cases of hairy cell leukemia variant all cases were negative for CD24 expression (0/7). In addition to the known unique phenotypic characteristics of HCL (i.e., CD11c-bright, CD19-bright, CD38-negative and CD123-positive), this study demonstrates that HCL is generally CD24-negative. Through routine evaluation of clinical PB and BM specimens we identified a low frequency memory B-cell subset exhibiting a similar phenotypic profile. These memory B-cells exhibit increased CD19 density and absent CD24 and CD38 expression. Hairy cell leukemia has been demonstrated to display a memory B-cell gene expression profile and a characteristic BRAF mutation. Examples of CD24 expression patterns in normal B-cell differentiation, mature memory B-cells and in HCL will be presented.

**Conclusions:** We show here that HCL and HCL variant are usually negative for CD24 expression. A memory B-cell subset exhibiting high-density CD19 in the absence of CD24 and CD38 expression can also be observed in normal PB and BM. The function of this normal CD24-negative memory B-cell and the relationship to the common, CD24-bright memory cells is unknown. The phenotypic profile suggests it represents the normal counterpart to hairy cell leukemia. Functional and gene profiling studies of this normal B-cell subset may provide additional insights into the biology of hairy cell leukemia.

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(28) RAPID DETECTION OF IMMUNOGLOBULIN HEAVY CHAIN GENE REARRANGEMENT BY PCR AND MELTING CURVE ANALYSIS USING COMBINED FR2 AND FR3 PRIMERS

Danfei Xu1*, Dongsheng Xu1, Zhuo Yang1, Wei Wu1, Ye Guo1, Wei Cui1
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**Introduction:** Immunoglobulin (Ig) gene rearrangement test is a standard tool in diagnosing B-cell lymphoma. The BIOMED-2 multiplex PCR protocol has become the most commonly used laboratory method for detection of monoclonal Ig heavy chain (IgH) rearrangement. However, the post-PCR procedure remains time-consuming and requires manual transfer. A novel strategy using LightCycler to continuously monitor fluorescence during melting curve analysis (MCA) can overcome these shortcomings. However, the previous studies on this method were all restricted to FR3 primers of BIOMED-2, which yield a low sensitivity. So we optimize the current method and include FR2 primers for the first time into MCA. **Methods:** FR2 primers were applied into MCA and combined to FR3 primers to detect IgH rearrangement (IgH-R) on LightCycler in 71 clinical DNA samples from formalin-fixed, paraffin-embedded tissues. There are 40 B cell non-Hodgkin lymphomas and 31 reactive lymphoid hyperplasia. We also test all lymphoma samples with identical FR2 primers by polyacrylamide gel electrophoresis (PAGE) and capillary gel electrophoresis (CE).

**Results:** MCA of combined FR2 and FR3 primer sets yields the sensitivity and specificity equal to 70% (28/40) and 100% (31/31), respectively. The addition of FR2 primers increases the sensitivity by 12.5% (5/40) comparing to FR3 primers alone (23/40). In comparison of three methods, two cases were positive in MCA, but negative in PAGE. These two samples were further tested by CE, which turned out consistent with MCA results. Detection of serial dilutions of Lymphoma cell line with the negative control at various percentages showed the analytical sensitivity of MCA was as high as 3.125%. **Conclusions:** This is the first study combining FR2 and FR3 primers for determining IgH-R by melting curve analysis in LightCycler system. It is high-throughput and faster. In addition, combined PCR and DNA melting curve analysis in a closed system reduce cross-contamination risk. Furthermore, it is more accessible than fragment analyzer for most hospitals in developing countries. Our method is slightly more sensitive to detect clonal IgH rearrangement compared to PAGE and comparable to CE. So we believe that PCR-MCA in the Light Cycler system has potential for evaluating monoclonal IgH-R in a clinical environment.
(29) DOUBLE CEBPA MUTATIONS, BUT NOT SINGLE CEBPA MUTATIONS, ARE PROGNOSTICALLY FAVORABLE IN PATIENTS WITH NON-M3 ACUTE MYELOID LEUKEMIA WITH WILD-TYPE NPM1 AND FLT3-ITD

Xiang-mei Wen, Jiang Lin, Zhao-qun Deng, Jing Yang, Dong-ming Yao, Chun-yan Tang, Gao-fei Xiao, Lei Yang, Ji-chun Ma, Jia-bo Hu, Wei Qian, Jun Qian
1Affiliated People's Hospital of Jiangsu Zhenjiang, China, 2Laboratory Center, Affiliated People's Hospital of Jiangsu University Zhenjiang, China, 3School of Medical Science and Laboratory Medicine, Jiangsu University Zhenjiang, China

Introduction: In the present study, we evaluated the frequency, the main associated features, and the prognostic significance of CEBPA mutations in a cohort of Chinese de novo non-M3 AML patients. Methods: The entire coding region of CEBPA gene was amplified by PCR and then sequenced in samples from 233 non-M3 AML patients. Results: Fifty mutations were identified in 37 (15.8%) patients with eleven (4.7%) double mutated CEBPA (dmCEBP A) and twenty-six (11.1%) single mutated CEBPA (smCEBP A). dmCEBP A was exclusively observed in M1 and M2 subtypes of FAB classification (P=0.008), whereas smCEBP A occurred in almost all subtypes (P=0.401). Patients with dmCEBP A had significantly younger age and higher WBC counts than those with wtCEBP A (P=0.016 and 0.043, respectively). Both dmCEBP A and smCEBP A were mainly present in cytogenetically normal patients. Patients with dmCEBP A achieved higher rate of complete (CR) than wtCEBP A patients (88% vs 51%, P=0.037), whereas smCEBP A and wtCEBP A groups are similar (47% vs 51%, P=0.810). Patients with dmCEBP A had a superior overall survival (OS) compared with patients with wtCEBP A (P=0.033), whereas patients with smCEBP A had a similar OS as patients with wtCEBP A (P=0.976). dmCEBP A but not smCEBP A was also associated with favorable outcome in patients with wild-type NPM1 and FLT3-ITD (NPM1™FLT3-ITD™). Conclusions: Our data confirm that dmCEBP A but not smCEBP A is prognostically favorable in NPM1™FLT3-ITD™ AML, and suggest that the entity AML with mutated CEBPA should be definitely designated as AML with dmCEBP A in WHO classification and smCEBP A should be excluded from the favorable risk of molecular abnormalities.

(30) JAK2 TRANSLOCATION, A RARE BUT RECURRENT THEME IN MYELOPROLIFERATIVE NEOPLASMS (MPNs)

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Introduction: Constitutive JAK2 activation via V617F mutation is highly prevalent in BCR/ABL1-negative MPNs. JAK2 activation by translocation is extremely rare. Its clinical and pathologic features are not thoroughly documented. Methods: Our molecular hematopathology database was queried for JAK2V617F-negative MPNs positive for 9p24 translocations. Two cases were identified and fluorescence in-situ hybridization (FISH) confirmed disruption of JAK2 in both. We studied their clinical, morphologic, and genetic features. CALR, MPL and qualitative BCR/ABL1 mRNA analyses were also performed. JAK2 fusion partner was evaluated by multiplex RT-PCR and sequencing. Results: Case 1: A 36-year old (yo) woman presented with marked granulocytic, left-shifted leukocytosis. Bone marrow (BM) resembled cellular phase primary myelofibrosis. Karyotype was complex including an unbalanced t(9;22)(p24;q11.2). FISH confirmed disruption of JAK2 in 86% of nuclei. Multiplex RT-PCR and sequencing identified 2 fusion transcripts: BCR exon 1 fused to JAK2 exon 15 and exon 17 respectively. The patient failed dasatinib therapy and underwent chemotherapy and allogeneic stem cell transplantation 4 months later. Case 2: A 38yo woman, diagnosed with unclassified MPN at 13 years of age, recently developed lymphadenopathy and hepatomegaly. Her peripheral blood smear showed a leukoerythroblastic picture. The BM revealed granulocytic hyperplasia with erythroid hypoplasia and left-shift and no distinctive megakaryocytic abnormalities. Karyotype was complex including an unbalanced t(8;9)(p22;p24) and fluorescence in-situ hybridization (FISH) confirmed disruption of JAK2 in 77% of nuclei. Retrospective FISH of a BM 7 years prior showed disruption of JAK2 in 16% of nuclei with a normal karyotype. Multiplex RT-PCR and sequencing of the latest BM showed 2 fusion transcripts: JAK2 exon 9 fused to PCM1 exon 36, and exon 36-exon 36’, respectively. JAK2V617F, CALR, MPL and qualitative BCR/ABL1 mRNA analysis were negative in both cases. All fusions were in-frame and involved the coiled-coiled domain in BCR and PCM1, and the JH1 tyrosine kinase (TK) domain and all/part of JH2 pseudokinase domain in JAK2, presumably leading to dimerization and constitutive activation of JAK2. Conclusions: JAK2 translocations are rare but recurrent in MPNs and have varying fusion partners and clinical/morphologic features. BCR-JAK2 cases may not respond to BCR/ABL1-targeting tyrosine kinase inhibitors. Further study of targeted therapy using JAK2 inhibitors in JAK2-translocated MPNs is warranted.
ORAL ABSTRACT SESSION: STANDARDS

(31) ASSEMBLY AND EVALUATION OF AN INVENTORY OF GUIDELINES THAT ARE AVAILABLE TO SUPPORT CLINICAL HEMATOLOGY LABORATORY PRACTICE

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Introduction: Practice guideline documents provide helpful support to the laboratory hematology community. A number of organizations have generated laboratory hematology guidelines. Our goal was to assemble an inventory of clinical practice guidelines focused on hematology laboratory topics. We postulated that this inventory would be a useful resource, and help assess for gaps in practice-focused guidelines.

Methods: An inventory of guidelines was assembled through searching the PubMed database and websites of organizations that support laboratory practice for public listings. Exclusion criteria included: annual reports, clinically-focused guidelines that covered laboratory tests but were mainly focused on diagnosis and management, scoring documents, collaborative study reports, position papers, guidelines on assays not performed in clinical laboratories, nomenclature documents, calibration documents and technical reports on standards.

Results: A total of 68 guidelines were identified that were focused on clinical laboratory hematology practice topics from organizations that included: Clinical and Laboratory Standards Institute (CLSI), International Society on Thrombosis and Haemostasis (ISTH), International Council for Standardization in Haematology (ICSH), British Committee for Standards in Haematology (BCSH), International Clinical Cytometry Society (ICCS), European Society for Clinical Cell Analysis (ESSA), American Association of Clinical Chemistry (AACC), College of American Pathologists (CAP), North America Specialized Coagulation Laboratory Association (NASCOLA), Institute for Quality Management in Healthcare (IQMH), European Molecular Genetics Quality Network (EMQN), and the World Health Organization (WHO). The median year of publication was 2010 and 15% were >10 years old.

The full scope of laboratory hematology practice was not covered by the inventory. Coagulation topics had the largest numbers of guidelines whereas some areas of practice had few guidelines. A minority of guidelines showed evidence of periodic updates, as some organizations did not remove or identify outdated guidelines.

Conclusions: This inventory of current practice guidelines will encourage awareness and uptake of guideline recommendations by the worldwide laboratory hematology community, with the International Society for Laboratory Hematology facilitating ongoing updates. There is a need to encourage best guideline development practices, to ensure that laboratory hematology community has current, high quality, evidence-based, practice guidelines that cover the full scope of laboratory hematology practice.

(32) SOURCE OF THE NORMAL DONOR PLASMA INFLUENCES THE DILUTE RUSSELL VIPER VENOM TIME CUT OFF VALUES FOR THE INVESTIGATION OF A LUPUS ANTICOAGULANT - ONE CENTRE’S EXPERIENCE.

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Introduction: International guidelines for the laboratory investigation of lupus anticoagulants (LA) recommend a minimum of 40 individual normal donor plasmas be used for the establishment of the cut off values for laboratory testing. Good laboratory practice requires laboratories to establish cut off values for each lot number of dilute Russell Viper Venom time (DRVVT) reagent prior to putting it into use for diagnostic laboratory testing. We observed that the source of the normal donor plasma influences the cut off value determined for the DRVVT test.

Methods: Frozen sodium citrate plasma was purchased from Precision Biologics (PB) (Dartmouth, Nova Scotia) and Affinity Biologicals (AB) (Ancaster, Ontario). 40 individual samples from each manufacturer were assayed. Samples were thawed in a 37°C waterbath as per manufacturer’s recommendations. LA Screen 1 (LA1) (low phospholipid) and LA Screen 2 (LA2) (high phospholipid) were purchased from Siemens Diagnostic Healthcare (Marburg, Germany) and reconstituted as per manufacturer’s recommendations. Testing was performed over 4 days on 3 Diagnostica Stago STAR/Evolutions (Asnieres, France).

Results: A total of 68 guidelines were identified that were focused on clinical laboratory hematology practice topics from organizations that included: Clinical and Laboratory Standards Institute (CLSI), International Society on Thrombosis and Haemostasis (ISTH), International Council for Standardization in Haematology (ICSH), British Committee for Standards in Haematology (BCSH), International Clinical Cytometry Society (ICCS), European Society for Clinical Cell Analysis (ESSA), American Association of Clinical Chemistry (AACC), College of American Pathologists (CAP), North America Specialized Coagulation Laboratory Association (NASCOLA), Institute for Quality Management in Healthcare (IQMH), European Molecular Genetics Quality Network (EMQN), and the World Health Organization (WHO). The median year of publication was 2010 and 15% were >10 years old.

When the 99th percentile cut off value was calculated, data confirmed that the cut off value for LA1 reagent differed significantly between manufacturers: PB=46 seconds; AB=52 seconds (p<0.05). A much smaller difference was noted with the LA2 calculated 99th percentile cut off value: PB=41 seconds; AB=42 seconds. The resulting LA1/LA2 ratio cut off value was significantly different between manufacturers: PB=1.20; AB=1.33 (p<0.05). When the data from both manufacturers were combined (n=80) the calculated cut off values for LA1=51 seconds; LA2=42 seconds and LA1/LA2 ratio=1.29.

Conclusions: Our observation illustrate that the source of normal donor plasma used to establish LA laboratory test cut off values has an influence on the calculated cut off value which could influence the classification of borderline or weakly positive LA samples. To limit these influences, we suggest that laboratories should use normal donor plasmas from multiple manufacturers to determine their LA cut off values and reference intervals.
PERFORMANCE IN BLOOD PARASITE EXTERNAL QUALITY ASSESSMENT

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Introduction: UK NEQAS for General Haematology has developed a two-stage scoring system for blood parasite external quality assessment that takes into account the participant’s service repertoire and expertise. Stage 1 scoring focuses on parasite screening only, is applicable to all participants and requires participants to record whether the case is positive or negative, and if positive, identify the parasite type: malaria, filaria, trypanosome, other, rather than provide a full species identification. Species identification for malaria parasites will be a required and assessed element of stage 2 scoring, for those laboratories that undertake this as part of their diagnostic service. Retrospective modelling of historic data has been undertaken to assess performance in stage 2 malarial parasite species identification.

Methods: The results of ten peripheral blood thin films were used for the historical performance modelling. The cases comprised six *P. falciparum*, one *P. vivax*, two *P. malariae* and one mixed *P. falciparum/P. ovale* infections, all confirmed by PCR testing. Two systems for malarial parasite species identification were modelled: the correct identification of all *Plasmodium* species (ALL_SP model) and the correct identification of *P. falciparum* vs. non-*P. falciparum* species (PF_ONLY model). False positive, false negative and incorrect parasite screening results were removed before modelling. An adverse penalty score was given for any result out of consensus with the target, using a look up table.

Results: 4552 results from 489 UK and international participants were assessed, using both the ALL_SP and the PF_ONLY models. When scored for agreement with the exact species present (ALL_SP model), as determined by PCR, 3600/4552 (79%) of results were as the target, 17 (0.4%) provided no species, 337 (7.4%) provided the correct plus one or more incorrect species, 309 (6.8%) misidentified *P. falciparum* and 289 (6.3%) misidentified another *Plasmodium* species. When scored for the identification of *P. falciparum* only (PF_ONLY model), 4204/4552 results were as the target, 22 (0.4%) were false positive for *P. falciparum* and 326 (7.2%) missed *P. falciparum*, including results where no species identification was provided. 104/489 (21%) laboratories were classified as persistent unsatisfactory performers using the ALL_SP model and 64 (13%) with the PF_ONLY model.

Conclusions: The high proportion of laboratories making multiple errors may reflect that not all of those included in the exercise will elect to undertake full species identification. The results indicate that full shadow scoring, at least of *P. falciparum* identification, is feasible, identifying laboratories requiring intervention to improve performance.

ADAPTATION OF KAPLAN MEIER ANALYSIS TO CHARACTERIZE HEMOGLOBIN TRANSFUSION TRIGGER KINETICS AT A LARGE TEACHING INSTITUTION IN ATLANTIC CANADA.

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Introduction: Understanding the impact of hemoglobin triggers on red blood cell (RBC) transfusion decisions can impact institutional blood inventory policies. To date, there has been no institution-wide characterization of the kinetics of hemoglobin triggers. We describe the adaptation of Kaplan Meier (KM) analysis, classically used to visualize the survival of populations, and apply it to visualize the probability by which hemoglobins are transfused.

Methods: De-identified inpatient data from January 2012-May 2014 queried from the laboratory information system at Capital District Health Authority, Nova Scotia was obtained. Data included hemoglobin values and RBC transfusions (occurring within 48 hours of hemoglobin result), including timestamps. Hemoglobin values were arbitrarily divided into the following groups: ≤40g/L, then 9 groups in 10g/L intervals and finally >130g/L. KM survival curves for the groups were constructed with time 0 being the time of hemoglobin measurement and the outcome being transfusion. No patient-specific identifiers were required.

Results: During the study period, there were 469,769 hemoglobin results. There were 25,188 units of blood transfused. Figure 1 represents the KM analysis of hemoglobin dropout secondary to transfusion. Table 1 summarizes the KM analysis according to transfusion probability at 48 hours and time to 50% transfusion.

Table 1: Break down of select hemoglobin group data. Note: Some groups were left out due to space limitation.

<table>
<thead>
<tr>
<th>Hb Level (g/L)</th>
<th>Number of Hb results (N)</th>
<th>Transfused at 48 hours (%)</th>
<th>Approximate time to 50% Transfusion (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤40</td>
<td>347</td>
<td>96.0</td>
<td>200</td>
</tr>
<tr>
<td>60&lt; and ≤70</td>
<td>14,640</td>
<td>86.0</td>
<td>600</td>
</tr>
<tr>
<td>70&lt; and ≤80</td>
<td>44,696</td>
<td>50.2</td>
<td>-</td>
</tr>
<tr>
<td>80&lt; and ≤90</td>
<td>61,319</td>
<td>23.5</td>
<td>-</td>
</tr>
<tr>
<td>&gt;130</td>
<td>112,437</td>
<td>1.1</td>
<td>-</td>
</tr>
</tbody>
</table>

Conclusions: Using KM analysis on hemoglobin values, we are able to determine the probability of transfusion at different hemoglobin levels. By understanding this, it will allow for the future development of a probabilistically driven algorithm for RBC unit ordering.
CONCURRENT 5: ICSH

(35) ICSH RECOMMENDATIONS FOR THE STANDARDIZATION OF PERIPHERAL BLOOD CELL MORPHOLOGY NOMENCLATURE AND GRADING

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Laboratory Hematology is continuing to gain strides in the standardization of its processes. Part of this is due to the increased use of automated rules for the review of the CBC and WBC differential, whether onboard the instrument, in the LIS or in Middleware. However, when the rules indicate that a peripheral smear must be performed, nomenclature and grading of the various cell abnormalities is often times subjective and inconsistent among laboratories in the same city and even within the same laboratory. While some regional guidelines exist, there is no one worldwide consensus for a nomenclature and grading system for WBCs, RBCs and Platelets. This lack of a global standard led the International Council for Standardization in Haematology (ICSH) to form an expert panel to investigate and recommend such guidelines. The panel is composed of sixteen members representing eleven countries. Many of the panel members have participated in establishing regional guidelines for morphology grading previously. These guidelines provide information on how to reliably and consistently report abnormal red blood cells, white blood cells and platelets using manual microscopy. Grading of abnormal cells, nomenclature and a brief description of the cells are provided in the final text, published in the International Journal of Laboratory Hematology (IJLH). It is important that all countries in the world use consistent reporting of blood cells. This issue is becoming more important as hospitals and laboratories join with others, forming large hospital and laboratory systems, with physicians practicing at multiple sites and with patients able to gain access to the health care system from multiple points of entry. There are also laboratories which are crossing international borders. Laboratory services can now span multiple countries. The need for consistent nomenclature and appropriate grading standards are therefore more important than ever. This international group of morphology experts has decided on these guidelines using consensus opinion. For some red blood cell abnormalities it was decided that parameters produced by the automated hematology analyzer might be more accurate and less subjective than grading using microscopy or automated image analysis and laboratories might like to investigate this further. The presence of qualitative flags and / or quantitative abnormal results may indicate the need for peripheral blood (PB) film review and / or a manual differential count. The examination of a well made and stained PB film coupled with the complete blood count information and the ability and skill of the reviewer adds qualitative and / or quantitative information and is an essential part of the diagnostic work-up. Abnormal morphologic findings are reported in various ways: i) a simple description, ii) the use of terms such as present or absent, iii) a semi-quantitative determination, mild (+), moderate (++), marked (+++), iv) a quantitative percentage of the morphological abnormalities: normal (<5%), mild (5-25%), moderate (25-50%), marked (>50%). Worldwide, there is marked variation in blood film evaluation, reporting practices and morphology terminology with recommendations in the literature and in local regional publications from a number of different national societies including the College of American Pathologists (CAP), the United Kingdom National External Quality Assessment Service (UK NEQAS), the Japanese Society for Laboratory Hematology and the Royal College of Pathologists of Australasia Quality Assessment Programs (RCPA QAP). Although there is no evidence that one reporting system is superior to the others, it has become evident that there is a need to develop a global consensus guideline for the grading of blood film abnormalities and blood film reporting as part of good laboratory and clinical practice and for use by laboratory accrediting agencies. The aim of the ICSH committee on Standardization of Peripheral Blood Cell Morphology, Nomenclature and Grading is to provide a guideline for the nomenclature and grading of red cell, white cell and platelet abnormalities.

References

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Bone marrow tissue biopsy (BMTB) evaluation by immunohistochemistry is performed on the trephine biopsy and/or clot section. This method allows for in situ evaluation of protein expression and is an important and integral part of bone marrow investigation. An international Working Party for the Standardization of Bone Marrow IHC was formed by the International Council for Standardization in Hematology (ICSH) in order to prepare a set of guidelines for the standardization of bone marrow IHC. The Working Party developed recommendations based on currently available published evidence and modern understanding of quality assurance principles as applied to immunohistochemistry in general. For pre-analytical component, narrowing choices for fixation and decalcification appears to be one of the most important mandates in the process of developing BM IHC standards. For analytical component, standardization refers to standardization of the performance characteristics, including sensitivity, specificity, reproducibility, and repeatability. For post-analytical component, we emphasize that the interpretation of the BM IHC results should be performed by the (hemato) pathologist familiar with the complexities of the bone marrow tissue processing and their potential effects for the IHC results, only after evaluation of the external and internal controls, which will indicate if the IHC test is properly calibrated and that no internal tissue factors prevent interpretation of specific IHC staining results and in conjunction with other available results (e.g. clinical history, other laboratory data, findings in the bone marrow aspirate smears and peripheral blood).

References


Hereditary spherocytosis, hereditary elliptocytosis, and hereditary pyropoikilocytosis are non-immune hereditary hemolytic anemias caused by the weakening of protein-protein interactions at different domains of the red cell cytoskeleton (e.g., at the junction where 2 or more membrane proteins cross-link with one another). The causal factor can be either a deficiency of or a qualitative defect in the constituent components band 3, ankyrin, protein 4.1, protein 4.2, and spectrin (αβ) proteins. Invoking a diagnosis of one of these red cell membrane disorders usually relies on finding spherocytes, elliptocytes or red cell fragmentation on blood smear of a patient presenting with a hemolytic condition together with or without a family history. The lifespan of circulating red cells in these patients is shortened due to splenic conditioning of the fragile red cells. The more severe is the hemolytic anemia in a patient, the greater the reduction of the red cell size. Hereditary stomatocytosis (HSt) is a collective name for a group of “under-reported”, autosomal dominant red cell membrane disorders. Their gain in attention in the last 15 years is due to the increased risk of venous thromboembolism post-splenectomy for dehydrated hereditary stomatocytosis. The following three subtypes are known to have a hemolytic condition: Dehydrated Hereditary Stomatocytosis (DHSt or xerocytosis) is the most prevalent form of HSt, followed by Overhydrated HSt, and Cryohydrocytosis (CHC). Familial Pseudohyperkalemia (FP) is found in individuals with apparently normal hematology. Stomatocytes are not always present on the blood smear of patients. Depending on the subtypes, stomatocytes or target cells, raised mean cell volume and mean cell haemoglobin concentration, together with elevated plasma potassium (indicating a passive K+ leak from the red cells) are helpful clues for identifying these patients. A different transmembrane protein is associated with each of the subtypes (namely, RhAG, Piezo 1, Band 3, Glut-1, and ABCB6).

All of the proteins reside in the outer membrane lipid bilayer of the cell which is shortened due to splenic conditioning of the fragile red cells. They are either transporter or sensory receptor with a role in regulating the cation flux of red cells. Functional impairment is manifested as an imbalance of the intracellular sodium (Na+) and potassium (K+) concentrations in the red cells. This Guideline (1) presents an integrated approach on how to identify a patient likely to have one of these red cell defects. That includes the characteristic red cell features (e.g., red cell morphology, red cell indices) and the exclusion criteria for each of the disorders. The range of currently available screening tests for the diagnosis of hereditary spherocytosis are also described together with the more complex tests (SDS-PAGE of membrane proteins and an assessment of molecular analysis of membrane protein genes) for the purpose of further investigation.

References

CONCURRENT 6: PLATELETS AND VON WILLEBRAND DISEASE

(38) VON WILLEBRAND FACTOR LABORATORY TESTING
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Von Willebrand disease (VWD) is considered the most common inherited bleeding disorder, and may also be the most difficult to diagnose. Clinical symptoms of VWD include predominantly mild mucosal bleeding; surgical bleeding may occur with specific challenges and joint bleeding can occur in the most severe forms. A family history of either diagnosed VWD or of bleeding symptoms is typically present. Laboratory diagnosis requires a series of assays of von Willebrand factor (VWF) function, with no single straightforward diagnostic test available to either confirm or exclude the diagnosis. VWF antigen (VWF:Ag) is a quantitative assessment of the plasma VWF protein level. While reliable and reproducible, when tested alone it does not evaluate VWF function. VWF ristocetin cofactor activity (VWF:RCO) is the current test to assess the ability of VWF to bind GPIbα. Several problems limit its reliability, including the high coefficient of variation and the fact that ristocetin is a non-physiologic agonist, but improved assays evaluating VWF- GPIbα interactions are under development. Factor VIII (FVIII) measurement is included in initial workup because VWF is a carrier protein for FVIII. Additional specialty tests may be performed to further elucidate the correct diagnosis. These include multimer distribution to document loss of high molecular weight multimers in types 2A and 2B VWD, platelet binding assays to document the gain of function defect in type 2B VWD, FVIII binding assays to document the decrease in FVIII binding found in type 2N VWD, and collagen binding, which is decreased in patients with loss of high molecular weight multimers and in patients with a specific defect in VWF-collagen binding. DNA sequencing may also be helpful to demonstrate specific gene mutations in VWF present in type 2 VWD, although novel variants should be interpreted with caution. Laboratory diagnosis is important for categorizing the specific type of VWD present, which will facilitate determining optimal treatment of affected patients.

References


(39) LABORATORY TESTING FOR PLATELET FUNCTION DISORDERS
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Platelet function testing is both complex and labour intensive. A stepwise approach to the evaluation of patients with suspected platelet disorders will optimize the use of laboratory resources, beginning with an appropriate clinical evaluation to determine whether the bleeding is consistent with a defect of primary hemostasis. Clinical bleeding assessment tools, evaluation of platelet counts, and review of peripheral blood cell morphology can aid the initial assessment. There is no ideal simple or sensitive screening test that reliably identifies patients requiring specialized testing of platelet function. Although both bleeding times and PFA-100/200® closure times have been used for this purpose, these tests are not adequately sensitive to rule out the need for further testing, and should be considered optional. A validated clinical bleeding assessment tool may be more useful in assessing a patient’s bleeding propensity and determining whether further specialized laboratory investigations are warranted. For patients requiring further laboratory testing, platelet aggregometry, secretion assays and von Willebrand factor assays are the most useful next steps, and will direct further specialized testing including flow cytometry, electron microscopy and molecular diagnostics. Guidelines and recommendations for standardizing platelet function testing, with a particular focus on light transmission aggregometry, are available and can provide a template for clinical laboratories in establishing procedures that will optimize diagnosis and assure quality results. The most widely used method of assessing platelet function is light transmission aggregometry, in which the change in optical density of a stirred sample of citrated platelet-rich plasma is measured by a photometer following the addition of agonists. Many pre-analytical and analytical variables affect the results. International surveys documented wide variations in testing parameters before the introduction of consensus guidelines. These guidelines provide recommendations on blood collection, preparation of platelet-rich plasma, testing parameters, agonist panels and interpretation of results. Whole blood aggregometry measures aggregation as the change in electrical impedance between two electrodes as platelets adhere and aggregate in response to agonists. It has the advantages of using smaller blood volumes and requiring less manipulation of the sample. Consensus recommendations for standardized testing are presently more limited than for light transmission aggregometry. The most useful additional assay in the clinical laboratory is a measure of platelet granule secretion. Secretion defects are relatively common, but often missed if standard light transmission aggregometry is the sole functional test. Lumi-aggregometry, in which the secretion of ATP from platelet dense granules is measured by the use of a luciferin/luciferase reagent, is the most widely used clinical assay, although other methods are available. The cause for most secretion defects cannot be identified in the clinical laboratory, but quantitation of platelet dense granules will identify a subgroup of patients with dense granule deficiency. Additional laboratory investigations including flow cytometry and genotyping can provide specific diagnoses in patients with defined abnormalities. Platelet function testing has presented unique challenges for quality assurance programs, because of the complexities of sample preparation and shipping. However, a number of organizations now offer external proficiency testing for evaluation of closure times, light transmission aggregometry, dense granule counting by electron microscopy, and clinical case interpretation.

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There are additional instruments/assays for point-of-care and near point-of-care testing of platelet function. The major focus of many of these instruments has been the monitoring of anti-platelet therapy in the setting of cardiovascular disease. Newer technologies, including microfluidics, hold the promise of changing how we study in vitro platelet function in the future.

References


(40) POLYPHOSPHATE, PLATELETS, AND COAGULATION

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Although the basic outline of the “waterfall” or “cascade” model of the blood clotting cascade has now been understood for more than fifty years, new players in the blood coagulation continue to be discovered. In particular, it is becoming increasingly clear that there are key connections between the blood clotting system and important biological processes such as inflammation and innate immunity. In 2006, our lab showed that polyphosphate, an inorganic polymer secreted by activated platelets and mast cells, is a modulator of the blood clotting system. We and others have now shown that polyphosphate accelerates blood clotting reactions and slows fibrinolysis, in a manner that is highly dependent on the polymer length of polyphosphate. Very long-chain polyphosphate (of the type present in microorganisms) is an especially potent trigger of the contact pathway of blood clotting, enhances the proinflammatory activity of histones, and may participate in host responses to pathogens. Polyphosphate also inhibits complement, providing another link between polyphosphate and inflammation/innate immunity. Platelet-size polyphosphate (which is considerably shorter than microbial polyphosphate) accelerates factor V activation, opposes the anticoagulant action of tissue factor pathway inhibitor, modulates fibrin clot structure, and promotes factor XI activation. Polyphosphate is a potential target for developing a new class of anti-thrombotic drugs with a novel mechanism of action and potentially fewer bleeding side-effects compared to conventional anticoagulants.

References

CONTINUOUS FLOW

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THROMBIN GENERATION AND FIBRIN FORMATION IN WHOLE BLOOD APPLYING CONTINUOUS FLOW

Introduction: Thrombin generation (TG) and fibrin formation (FF) do not always go hand in hand; for example factor XIIa influences the crosslinking of fibrin independently of thrombin. We aimed to validate an assay to simultaneously measure TG and FF in whole blood under conditions of flow. Methods: We have redesigned an air-bearing rheometer, rendering it sensitive to measure FF based on changes in viscosity. Introduction of a fluorescent camera allowed the measurement of TG based on the conversion of a thrombin-sensitive substrate, while applying laminar flow conditions within the range of 100 to 1200s⁻¹. Results: For all variables the intra-/inter-assay variation was below 10% in platelet-poor plasma (PPP). For endogenous thrombin potential (ETP), peak and maximum clot strength (viscosity) we demonstrated an inverse relationship with increasing flow rates in PPP. The addition of increasing concentrations of fibrinogen to defibrinated plasma resulted in an increase in ETP, peak and maximum clot strength. In 70 patients undergoing cardiothoracic surgery, TG parameters in blood samples taken pre-bypass correlated with calibrated automated thrombinography parameters, whereas FF correlated with fibrinogen content and rotational thromboelastometry. Upon dividing patients into two groups based on the median clot strength, a significant difference in perioperative/total blood loss was established. In a separate study, this technique revealed reduced fibrinolysis at venous shear rate in infants and children compared to adults and this difference was much less pronounced at arterial shear rate. Conclusions: Our method enables the simultaneous measurement of TG and FF in plasma/whole blood under physiological conditions of continuous flow and is sensitive to differences in fibrinogen content. Interestingly, FF proved to be indicative for the amount of blood loss post cardiothoracic surgery and corresponded with the lower incidence of thrombosis in infants and children compared with adults.

METHOD FOR AUTOMATIC RECOGNITION OF NEOPLASTIC LYMPHOID CELLS USING PERIPHERAL BLOOD CELL IMAGES

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Introduction: Morphologic differentiation of neoplastic lymphoid cells in peripheral blood (PB) is a difficult task, which requires high experience and skill due to subtle morphologic characteristics exhibited by these cells. This work aims to apply a method to automatically classify several types of lymphoid cells using PB digital images, including normal lymphocytes (N), reactive lymphoid (RL) cells and neoplastic lymphoid cells. Methods: We analyzed a total of 3496 lymphoid cell digital images from PB films stained with May Grünwald-Giemsa and obtained in the CellVision DM96. From them, 260 images were normal lymphocytes and the remaining images were 529 hairy cell leukemia (HCL) cells, 863 Chronic Lymphocytic Leukemia (CLL) cells, 732 Mantle Cell Lymphoma (MCL) cells, 551 Follicular lymphoma (FL) cells, 214 B-cell Prolymphocytes (BPL) and 347 RL. We segmented 3 regions by using color clustering: nucleus, cytoplasm and peripheral cell region. Then, we extracted from these regions 13 geometric and 6422 color-texture features from multiple color spaces. Subsequently, we applied information theoretic feature selection to determine the 55 most relevant and less redundant features. They were used to build a support Vector Machine (SVM) classifier with a radial basis function kernel to automatically recognize the lymphoid cell subtypes. Results: The automatic recognition system was evaluated by implementing a 10-fold cross-validation using the SVM classifier. The classification average accuracy of this procedure was 92.07% , with true positive classification rates (TPR) of 95.08% for HCL, 95.60% for CLL, 90.93% for FL, 90.98% for MCL, 87.85% for BPL cells, 85.83% for N and 91.64% for reactive lymphocytes. Figure 1 shows the positive predictive value (PPV), the true positive rate (TPR) and the true negative rate (TNR) for each lymphoid cell subtype in the evaluation process. Conclusions: The system described was able to automatic classify the following 7 different groups of lymphoid cells in PB: normal, reactive lymphocytes and 5 subtypes of B neoplastic lymphoid cells. As the best of our knowledge, the automatic recognition of such high number of different lymphoid cells has not been previously published. We are progressing with further work to include other B and T cell leukemias and blastic cells to design a practical diagnosis support tool.

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<td>HCL</td>
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<td>87.85</td>
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<td>CLL</td>
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(43) EPIGENETIC-REGULATOR AND SPlicING-GENE MUTATIONS IN ACUTE MYELOMONOCYTIC LEUKEMIA (AMML) AND ACUTE MONOCYTIC LEUKEMIA (AMoL)

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1Xin Hua Hospital Shang hai, China, 2Oregon Health & Science University Portland, OR, USA

Introduction: Mutations in epigenetic-regulators and splicing-genes have been implicated in the pathogenesis of acute myeloid leukemia (AML). Next Generation Sequencing (NGS) can simultaneously detect multiple mutations in leukemia. Mutation screening in AML is an integral part of risk stratification to guide therapy and monitor disease response/relapse. We report mutations in AMML/AMoL using targeted NGS. Methods: The study included 16 AMML and 4 AMoL from the pathology department/Knight diagnostic laboratory/Oregon Health and Science University (OHSU) (2013-2014). The targeted-NGS-panel covered 42 genes relevant to hematopoietic malignancies (Table 1). Sequencing required 20ng of DNA from patients using Ion-Torrent-PGM. Bioinformatics-analysis was performed by Torrent-Suite-v.3.2 -pipeline (Life-Technologies, CA). Open-source programs and lab-developed algorithms were used for annotation and amino acid prediction. Results: As shown in Figure1, mutations in epigenetic-regulators (DNMT3A, IDH, TET2) and splicing-genes (U2AF1, SRSF2, SF3B1) were common in AMML/AMoL: 50% (10/20) of all cases including 100% (9/9) of cytogenetically-normal (CN) and only 17% (1/6) of complex cytogenetics cases. Among all epigenetic-pathway mutations, DNMT3A was the most frequently mutated (60%, 6/10) and often occurred with FLT3-ITD (3/6, 50%) and NPM1 (6/6, 100%) mutations, but was mutually exclusive with other epigenetic-pathway genes. Table 2 illustrates one case which NGS was performed 3 times: three gene mutations (FLT3-ITD, NPM and DNMT3A) were detected at high allele frequencies at diagnosis (day 0) and relapse (day 92). Only the DNMT3A mutation persisted in the remission marrow (day 28).

Table 1: Targeted-NGS-Panel

<table>
<thead>
<tr>
<th>Epigenetic-regulator</th>
<th>IDH1, IDH2, DNMT3A, TET2, EZH2, ASXL1, KDM6A/UTX, SUZ12</th>
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<tbody>
<tr>
<td>Splicing-genes</td>
<td>SF3B1, SRSF2, ZRSR2, U2AF1</td>
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<tr>
<td>Kinase</td>
<td>FLT3, KIT, JAK1, JAK2, JAK3, ABL1</td>
</tr>
<tr>
<td>Signaling-molecules/others</td>
<td>PTPN11, MPL, IL-7R, CSF3R, CBL, CBLB, NRAS, KRAS, HRAS</td>
</tr>
<tr>
<td>Transcriptional-factor</td>
<td>GATA1, GATA2, CEBPA, ETF6, RUNX1, STAT3, PAX5, IKBKAP</td>
</tr>
<tr>
<td>Tumor-suppressor/others</td>
<td>TP53, WT1, FBXW7, CREBBP, NOTCH1, BCO1, NPM1</td>
</tr>
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</table>

Table 2. A patient with AMML

<table>
<thead>
<tr>
<th>Date</th>
<th>Progress</th>
<th>Cyogenetic</th>
<th>FLT3-ITD</th>
<th>NPM1</th>
<th>DNMT3A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day-0</td>
<td>Initial-Diagnosis(Blst=80%)</td>
<td>Normal</td>
<td>FLT3-ITD/ (40%)</td>
<td>p.W288fs*12/ (40%)</td>
<td>p.V663M/ (94%)</td>
</tr>
<tr>
<td>Day-28</td>
<td>End-of-induction(Blst=3%)</td>
<td>Normal</td>
<td>undetectable</td>
<td>undetectable</td>
<td>p.V663M/ (20%)</td>
</tr>
<tr>
<td>Day-92</td>
<td>Relapse(Blst=30%)</td>
<td>Normal</td>
<td>FLT3-ITD/ (15%)</td>
<td>p.W288fs*12/ (10%)</td>
<td>p.V663M/ (40%)</td>
</tr>
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Conclusions: The study shows that mutations in epigenetic-regulators and splicing-genes are common in AMML/AMoL, which always coexist with other mutations, such as FLT3-ITD and/or NPM1, etc. especially in the CN-group. One case demonstrated that DNMT3A mutation persisted in the remission marrow when other mutations became undetectable. Our results support that DNMT3A occurs early in tumorigenesis and may cause genetic instability to induce FLT3, NPM1 mutations. Furthermore, epigenetic-regulators and splicing-genes are not sufficient for progression to acute leukemia and additional genetic “hits” are necessary. NGS is a powerful tool for multiple-mutation-analysis in the clinical-management and provides a better understanding of leukemogenesis.
A SINGLE-STEP WHOLE BLOOD MICRO ASSAY FOR THE RAPID DETECTION OF VON WILLEBRAND DISEASE
Marije Baaij1, Roger Schutgens1, Britta Laros-van Gorkom2, Waander van Heerde3, Wailung Leung1, Yvonne Sanders3, Frank Leebeek1, Philip de Groot1, Rolf Urbanus1, Mark Roest1,4
1UMC Utrecht Utrecht, Netherlands, 2St. Radboud University Medical Center Nijmegen, Netherlands, 3Erasmus University Medical Center Rotterdam, Netherlands, 4Synapse B.V. Maastricht, Netherlands

Introduction: The current diagnostic work up of Von Willebrand Disease (VWD) is laborious, has a high variability, poor precision and low reproducibility. For a reliable diagnosis of VWD, we need a rapid test to measure both qualitative and quantitative VWF defects. Methods: We have developed a single step assay to diagnose Von Willebrand disease with just 40 μl of unprocessed whole blood. The test was validated in fifty-six VWD patients with different hereditary subtypes and 60 healthy subjects. Ristocetin induced VWF-platelet interaction was measured with flow cytometry. Results: All VWD patients had lower ristocetin-induced VWF binding to platelets than healthy subjects (sensitivity and specificity 100%). Type 2B patients were identified with a 100% sensitivity and 99.1% specificity. Ristocetin-induced VWF binding was virtually absent in type 3 patients. Conclusions: We introduce a novel whole blood test to diagnose VWD. The test is reliable, has an excellent performance, a short turn-around-time and is easy to handle. The rapid evolution of next generation flow cytometers, facilitates a quick introduction of this test in routine diagnostic labs.

USING EVIDENCE FOR HEMATOLOGY LABORATORY PRACTICE
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Health Information Research Unit, Dept of Clinical Epidemiology and Biostatistics
Hamilton-Niagara Bleeding Disorders Program, Dept of Medicine
McMaster University, Hamilton, ON, Canada

Laboratory medicine has a central role in the generation and application of evidence. The key contribution is in the provision of properly validated and appraised diagnostic tests and algorithms, which however do not play an isolated role in research and clinical practice: the evidence based classification of diseases and the measurement of outcomes are critical to the progress of knowledge on the effectiveness of interventions, and a requisite for delivering evidence based care. Moreover, proper stratification of patients provides precious prognostic information, needed for care and informed life planning in many clinical situations. Over time, the awareness of the centrality of diagnosis and laboratory medicine in the generation of health evidence has been continuously increasing. An often-understated implication of the profound interconnection between diagnosis, prognosis and treatment is the need for close collaboration between the laboratory and the clinical parties in the assessment of the diagnostic properties of tests and algorithms.

Using examples from the published literature in laboratory hematology, we will review a few basic concepts of the application of EBM to laboratory medicine, the role for instruments like QUADAS2 and AGREE, and discuss the GRADE approach to issuing evidence based and transparent guidelines.
CONCURRENT 7: NEW ORAL ANTICOAGULANTS: LABORATORY CONSIDERATIONS

(46) QUANTIFYING AND ESTIMATING NOAC IN THE ROUTINE CLINICAL LABORATORY

Robert C Gosselin, CLS
UC Davis Health System
Department of Pathology and Laboratory Medicine
Sacramento, CA

Non-vitamin K oral anticoagulants (NOACs) are being used with increasing frequency due to their safety profile and ease of use, given that therapeutic monitoring is not required. As these agents have only recently been FDA approved, their effect on routine and specialty coagulation assays is not well appreciated. This presentation will address NOAC effect on routine coagulation assays and whether these assays can be used to determine relative concentration as well as alternative strategies to estimate or quantitate drug concentration in plasma. Also reviewed is the use of manufactured drug calibrators to determine reagent responsiveness and the effect of these agents on various special coagulation assays.

References

(47) LUPUS ANTICOAGULANTS: CHALLENGES AND NEW GUIDELINES

Thomas L. Ortel, MD, PhD
Duke University Medical Center

Antiphospholipid antibodies may be encountered in patients with venous and/or arterial thromboembolic events, recurrent pregnancy morbidity, or as an incidental finding in an otherwise asymptomatic individual. Antiphospholipid antibodies that can be associated with adverse clinical outcomes include lupus anticoagulants, diagnosed by coagulation assays, and anticardiolipin antibodies and anti-β2-glycoprotein I antibodies, diagnosed by serologic assays. Lupus anticoagulants can be difficult to detect and confirm, particularly in patients with additional coagulopathic disorders and/or on anticoagulant therapy. Updated guidelines have recently been published by the International Society on Thrombosis and Haemostasis (ISTH) and the British Committee for Standards in Haematology (BCSH), and the Clinical and Laboratory Standards Institute (CLSI) published its first guideline on lupus anticoagulants in 2014. This presentation will review these guidelines and discuss where they agree and where they differ, and will propose strategies that can be taken to improve diagnostic accuracy for these clinically relevant autoantibodies.
(48) CHALLENGING HEMOSTASIS SCENARIOS IN PEDIATRIC PATIENTS—A CASE STUDY-BASED DISCUSSION

Karen A. Moser, M.D.
Saint Louis University School of Medicine
Department of Pathology

Pediatric patients differ from adult patients in many clinical situations, and disorders of hemostasis and thrombosis are no exception. This educational session will present clinical and laboratory features of two cases in which pediatric patients are evaluated for bleeding disorders. Discussion of the cases focuses on practical considerations for laboratorians. Review of these case studies also highlights selected common and esoteric issues in pediatric hemostasis testing.

References


CONCURRENT 8: LABORATORY TESTING FOR HEMATOLOGICAL MALIGNANCIES

(49) MOLECULAR ADVANCES IN THE DIAGNOSIS AND CLASSIFICATION OF MYELOPROLIFERATIVE NEOPLASMS

Adam Bagg, MD
Department of Pathology and Laboratory Medicine
University of Philadelphia

The myeloproliferative neoplasms (MPNs) include the so-called classical MPNs of chronic myelogenous leukemia (CML) and the non-CML MPNs, polycythemia vera (PV), essential thrombocytemia (ET) and primary myelofibrosis (PMF). The molecular genetic basis of CML has been known for some time, while that of PV, ET and PMF has only been dissected in the past decade. With this understanding of the primary mechanisms that generate these MPNs has come a quest to develop more specific diagnostic assays, and more specific molecularly targeted therapy. This is best exemplified in CML, in which the BCR-ABL1 oncoprotein has been demonstrated to be pivotal, with its inhibition by tyrosine kinase inhibitors (TKIs) resulting in dramatic responses leading to the functional cure of this once frequently fatal disease. Further, the t(9;22) Philadelphia chromosome-generating BCR-ABL1 fusion gene, the documentation of which is essential for the diagnosis of CML, has proved to be a rational and convenient target to monitor to determine the efficacy of therapy. The discovery of JAK2 mutations (and others, including those affecting CALR and MPL) in variable proportions of the so-called Philadelphia chromosome-negative MPNs of PMF, PV and ET have been fascinating biologically and invaluable diagnostically. However, it has also emerged that the CML/BCR-ABL1 paradigm, both in terms of disease genesis and therapeutic response monitoring, cannot (yet) be applied to these other MPNs. Indeed, it has emerged that the genetic basis of these MPNs is much more complex than just the presence of these mutations, and current JAK2 inhibitors, for example, while providing some salutary clinical responses, do not appear to have as dramatic effects on the levels of the mutated gene (less than a log decrease), as compared with the over 3 logs, and often greater, decrease seen in the context of BCR-ABL1 inhibitors. A plethora of mutations has also been detected in some non-classical MPNs (such as CSF3R mutations in chronic neutrophilic leukemia) and in other neoplasms currently somewhat awkwardly classified as mixed myeloproliferative neoplasms/myelodysplastic syndromes. The latter include SETBP1 mutations in atypical chronic myeloid leukemia, RAS pathway mutations in juvenile myelomonocytic leukemia (including those affecting NF1, NRAS, KRAS, PTPN11 and CBL) and ASXL1 mutations (amongst many others) in chronic myelomonocytic leukemia. These discoveries play a pivotal role in diagnosis and/or prognosis. In summary, the clinical laboratory, primarily using molecular genetic approaches, plays an ever-expanding role in the contemporary diagnosis, prognostic stratification and monitoring (best established for CML) of MPNs and related neoplasms.

References

The diagnosis and classification of lymphoid neoplasms has evolved significantly over the past few decades with the advances in the understanding of pathogenesis of various lymphoid malignancies. Clinically, there has been an increasing emphasis on the personalized treatment approach to lymphoid neoplasm due to the biological heterogeneity within a specific entity. This presentation will focus on the immunohistochemical/immunophenotypic or molecular markers discovered in the last few years that have diagnostic application for lymphoid neoplasms. A definitive diagnosis of mantle cell lymphoma (MCL) often requires correlation of morphologic findings with immunophenotype, cyclin D1 expression by immunohistochemical study or t(11;14)-CCND1 and IgH fusion by FISH or cytogenetic analysis. In the last several years, the overexpression of transcription factor SOX11 has been found to be a highly reliable immunohistochemical marker for MCL among small B-cell lymphomas, and is particularly useful for recognizing cyclin D1-negative MCL. An indolent variant of MCL has drawn increasing attention in recent years, and this variant is characterized by blood and bone marrow involvement with minimal nodal disease. SOX11 was reported to be negative in this indolent variant of MCL. However, it remains to be determined whether SOX11 could be used as a prognostic indicator for MCL. Chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL) is the most common B-cell leukemia in the western countries, and its diagnosis can often be made based on the morphologic and immunophenotypic characteristics. However, when CLL/SLL displays an atypical morphology or immunophenotype, particularly in a peripheral blood sample or small biopsy specimen, the diagnosis could be challenging. Recently, the overexpression of LEF1, a component of nuclear complex involved in the beta-catenin signaling pathway, has been found to be a highly specific immunohistochemical marker for CLL/SLL and helps differentiating CLL/SLL from other small B-cell lymphomas such as MCL and CD5+ marginal zone lymphoma (MZL). Recently, flow cytometric analysis has been successfully applied to the evaluation of SOX11 and LEF1 in clinical samples, and this is particularly useful in the evaluation of B-cell neoplasms with leukemic presentation. Additionally, a membrane glycoprotein, CD200, has been found to be uniformly expressed in CLL/SLL and is absent in MCL, MZL and follicular lymphoma (FL). Detection of CD200 expression by immunohistochemistry or flow cytometry helps distinguishing between CLL/SLL and MCL. Several new molecular abnormalities associated with specific subtypes of lymphoid neoplasm have been recently uncovered, and these molecular abnormalities not only provide insights into the pathogenesis of the lymphoid neoplasms, but also provide diagnostic markers and potential therapeutic targets for the diseases. A recurrent somatic mutation, MYD88 L265P, has been identified in over 90% of lymphoplasmacytic lymphoma/Waldenstrom macroglobulinemia (LPL/WM), and the mutation is only present in a small percentage of splenic marginal zone lymphoma and absent in plasma cell myeloma. Thus, PCR analysis of MYD88 L265P helps with the differential diagnosis between LPL/WM and its mimics when combined with clinical and morphologic evaluation. Assessment of MYD88 L265P may also be useful in monitoring disease progression and assessment of minimal residual disease (MRD) in patients with LPL/WM. Hairy cell leukemia (HCL) is an indolent disease that is sensitive to purine analog therapy. A definitive diagnosis of HCL often relies on the correlation of morphologic findings with the typical immunophenotype. Recently, a heterozygous mutation in the BRAF gene, BRAF V600E, has been identified in almost all HCL cases, and is highly specific for HCL among hematologic malignancies. Diagnostic testing for BRAF mutation is available in clinical laboratories. Therapeutics targeting the V600E BRAF mutant has shown effectiveness in patients with refractory HCL. T-cell large granular lymphocytic leukemia (T-LGLL) is an indolent disease and often presents with severe neutropenia with or without anemia. The distinction between reactive T-LGL proliferation and T-LGL can be difficult in some cases. Additionally, patients with T-LGL may not have T-LGL lymphocytosis in the peripheral blood despite apparent bone marrow involvement. Recent studies have demonstrated that STAT3 mutations in 30 to 40% T-LGL with recurrent mutational hot spots. Thus, testing for STAT3 mutations may distinguish true neoplastic proliferation from reactive expansion of T-LGLs. Many advances have been made in recent years in better understanding of the pathogenesis of various lymphoid neoplasms. Incorporation of the analysis of the newly uncovered, subtype-specific immunohistochemical/immunophenotypic or molecular markers in the clinical workup of patients with lymphoid neoplasms will certainly improve the diagnostic accuracy. It may also provide important prognostic information and therapeutic targets for newly developed drugs.

References

(51) ADVANCES IN THE PATHOGENESIS AND DIAGNOSIS OF MULTIPLE MYELOMA
P. Leif Bergsagel, MD
Mayo Clinic Arizona

Multiple myeloma is a tumor of indolent, bone marrow localized, isotype-switched plasma cells. Recently the diagnostic criteria have been amended to include some patients who would previously have been diagnosed with ultra-high-risk smoldering multiple myeloma and benefit from immediate treatment. Genetically it can be divided into tumors with different recurrent oncogenes or tumor suppressor genes. Recent genomic studies have shown that almost half of untreated patients have a genetic rearrangement of the MYC locus that result in juxtaposition of ectopic super-enhancers adjacent to MYC, as well as somatic mutations that activate the RAS/MAPK pathway (NRAS, KRAS, BRAF, FGFR3). Mutations that result in constitutive activation of the NFkB pathway and that inactivate TP53, CDKN2C, KD-M6A, FAM46C, DIS3 are also recurrent. A major insight from these studies has been the recognition of the high degree of subclonal heterogeneity in multiple myeloma, which is more frequent in patients with high-risk genetics. The subclones may alternate in dominance under alternating therapeutic pressure, a phenomenon known as “clonal tides”. The identification of marked subclonal heterogeneity argues in those patients for the use of therapeutic strategies to maximize response, and long-term suppressive therapies to prevent tumor re-growth and development of additional sub clones.

References


CONCURRENT 9: ANEMIAS AND RED CELL DISORDERS

(53) DISORDERS OF ERYTHROCYTE VOLUME
Patrick G. Gallagher, MD
Pathology and Genetics, Yale University School of Medicine
Yale Center for Blood Disorders

Disorders of erythrocyte volume homeostasis are a heterogeneous group of inherited disorders with phenotypes ranging from dehydrated to overhydrated erythrocytes. These disorders are classified by the degree of abnormal membrane permeability to sodium and potassium, resulting in concomitant alteration of intracellular water. Depending on the degree of perturbation of water and solute homeostasis and its effect on the erythrocyte, hemolytic anemia of varying severity may occur. Historically, these disorders were called the stomatocytosis syndromes due to the variable numbers of stomatocytes sometimes observed on peripheral blood smear. However, stomatocytosis is rare in some of these disorders and it is not a sine qua non for diagnosis. With these previous observations in mind, it is not surprising that genetic bases of the erythrocyte volume homeostasis disorders are now proving to be heterogeneous, as they parallel the marked variability in clinical, laboratory and physiologic manifestations observed from patient to patient. Disorders of erythrocyte volume homeostasis have been classified as primary, due to inherent disorders of volume regulation, and secondary, due to other disorders affecting the erythrocyte that also influence cell volume. Primary disorders with erythrocyte dehydration are the hereditary xerocytosis syndromes, while secondary erythrocyte dehydration is associated with spherocytosis, thalassemia, sickle cell disease, hemoglobin C disease, Southeast Asian ovalocytosis, and malaria invasion. In these disorders, the secondary erythrocyte dehydration is frequently a factor in disease pathobiology. Recent studies have implicated roles for PIEZO1, a long sought after mammalian mechanosensory protein, and SLC4A1, the anion transporter with implications for transfusion practice. Transfusion 54:3043-50


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CONGENITAL SIDEROBLASTIC ANEMIA
Mark Fleming
Harvard Medical School, United States

The congenital sideroblastic anemias (CSAs) are an uncommon, diverse class of inherited hematopoietic disorders characterized by pathological deposition of iron in the mitochondria of erythroid precursors. In recent years, the genetic causes of several clinically distinctive forms of CSA have been elucidated, which has revealed common themes in their pathogenesis. In particular, most, if not all, can be attributed to disordered mitochondrial heme synthesis, iron-sulfur cluster biogenesis or pathways related to mitochondrial protein synthesis. This review will summarize the clinical features, molecular genetics, and pathophysiology of each of the CSAs in the context of these pathways.

CONGENITAL DYSERYTHROPOIETIC ANEMIA TYPE IV AND OTHER HEMATOLOGIC PHENOTYPES ASSOCIATED WITH KLF1 GENE MUTATIONS
John S. Waye, PhD
Department of Pathology and Molecular Medicine, McMaster University, Hamilton, Ontario, Canada

Krüppel-like factor 1 (KLF1) is an erythroid-specific transcription factor that plays important roles in globin gene switching, erythroid lineage commitment and erythrocyte maturation. The KLF1 gene is located on chromosome region 19p13.2, spans approximately 3 kb of genomic DNA, and consists of three exons encoding a 362 amino acid protein. KLF1 contains three zinc fingers homologous to those found in the Krüppel family of transcription factors. KLF1 has two functional domains: a proline-rich amino-terminal activation domain and a carboxy-terminal domain that recognizes the consensus binding site motif found in the regulatory regions of many erythroid genes. KLF1 can act as both a transcriptional activator and repressor of erythroid gene expression, with preferential activation of genes for heme and globin synthesis, blood group antigens and other erythroid factors. Not surprisingly, the downstream effects of KLF1 mutations can result in a wide range of hematologic phenotypes, as reviewed in this presentation. Wickramasinghe and colleagues first described an atypical Congenital dyserythropoietic Anemia (CDA) phenotype in a Danish girl born to healthy non-consanguineous parents [1]. The proband was hydropic and severely anemic at birth, necessitating repeated transfusions during the first year, and only occasional transfusions thereafter. The phenotype was one of chronic normocytic anemia, prominent anisocytosis, elevated levels of Hb F (>40%), marked normoblastic erythroid hyperplasia, reticulocytosis and hyperbilirubinemia. Subsequent studies demonstrated dysregulation of globin gene expression, with embryonic ζ- and ε-globin chains present in some of her circulating erythrocytes [2]. The underlying genetic cause of this atypical CDA phenotype was later determined by Arnaud et al [3] who investigated the original patient and a second unrelated patient. They hypothesized that the underlying genetic defect could involve an erythroid transcription factor, and subsequently found that both patients were heterozygous for a de novo missense mutation of the KLF1 gene (c.973G>A, p.Glu325Lys) [3]. This mutation is predicted to stabilize binding of KLF1 to its DNA target sequences, acting in a dominant-negative manner to reduce transcription [3]. To date, there have been a total of four reported cases of this rare CDA, all heterozygous for the c.973G>A mutation [4], thus defining the CDA type IV (CDAN4, OMIM 613673). It was recently demonstrated that compound heterozygosity for loss-of-function KLF1 mutations can result in severe transfusion-dependent hemolytic anemia [5]. The key features were transfusion-dependent anemia associated with red cell pyruvate kinase deficiency, abnormalities in red cell membrane proteins, and globin gene dysregulation characterized by marked elevated Hb F levels and expression of embryonic globin genes into adulthood. All patients had genotypes that included at least one missense mutation, indicating that the condition is due to severe deficiency and not ablation of KLF1 function. There have been no reported homozygotes for KLF1 null mutations in humans, suggesting that this genotype may be embryonic lethal. Recent studies have demonstrated that KLF1 gene mutations are relatively common, particularly in populations where thalassemia is endemic. Simple carriers of loss-of-function mutations are healthy but may exhibit one or more benign hematologic phenotype including (i) hereditary persistence of fetal hemoglobin (HPFH), (ii) borderline elevated levels of Hb A2, (iii) marginal microcytosis and/or hypocho- roemia, and (iv) the rare In(Lu) blood group. KLF1 gene testing may be indicated for any of these conditions to explain the abnormal
phenotype and thus prevent unnecessary investigations, as well as to identify couples that might benefit from genetic counseling. This is particularly important given severe phenotypes associated with severe KLF1 deficiency.

References

High-dimensional flow cytometry is unquestionably the leading technology for cellular analysis because it allows for the simultaneous detection of numerous cellular characteristics on individual cells. Although this technology has become an invaluable tool in Drug Development, Translational Science, and Clinical Laboratories, there is a surprising lack of official validation guidelines specific to flow cytometry. Due to the challenges associated with cellular measurands, the lack of reference materials, and the complexity of the instrumentation, guidelines appropriate to other methodologies cannot fully be applied to flow cytometry. Thus, key stakeholders from both the pharmaceutical and clinical sectors have put forth considerable effort to generate recommendations for the optimization, validation, implementation, and monitoring of flow cytometric methods. In this presentation, the recommendations from the American Association of Pharmaceutical Scientists, International Council for Standardization of Haematology and the International Cytometry Society, will be reviewed. The key aspects of instrument validation, method development, and analytical method validation will be reviewed. In addition, the responses from international organizations to the 2014 FDA issued a draft guidance documents for flow cytometry and LDTs will be discussed.

References


Results between different clinical laboratory measurement procedures should be comparable, within clinically meaningful limits, to enable optimal use of clinical guidelines for diagnosis and patient management. The ISO standard 17511:2003 “In vitro diagnostic medical devices - Measurement of quantities in biological samples - Methodological traceability of values assigned to calibrators and control materials” describes a hierarchy of calibration traceability schemes to accomplish harmonization of results. The most desirable and best developed approaches for calibration traceability utilize primary (pure substance) reference materials to prepare calibrators for high level reference measurement procedures. The Joint Committee for Traceability in Laboratory Medicine maintains lists of reference materials, reference measurement procedures and laboratories performing reference measurement procedures that conform to the ISO standards for these reference system components. The ISO standard for traceability provides for the situation when there is no reference measurement procedure and traceability is to a secondary (matrix) reference material. However, inadequate attention to the commutability of secondary reference materials has led to the situation when clinical laboratory procedures are traceable to a reference material, yet results for patient samples are not equivalent when measured with different routine laboratory procedures. In addition, inadequate definition of the measurand (the quantity intended to be measured) and inadequate analytical selectivity for the measurand contribute to lack of harmonized results. For many measurands, secondary reference materials are not available, and alternative processes based on panels of patient samples are needed to achieve harmonization. Despite many organizations in many countries addressing harmonization, there is no systematic approach to prioritize measurands based on medical importance and to coordinate the efforts of different groups. An International Consortium for Harmonization of Clinical Laboratory Results has been formed to provide a global infrastructure to enable a systematic approach for identification and prioritization of measurands to be harmonized, an information portal to foster collaboration among all organizations contributing to harmonization of measurands, and a technical focus on measurands that do not have reference measurement procedures.

References

POSTER SESSION 1

100 - CELLULAR ANALYSIS
200 - COAGULATION
300 - FLOW CYTOMETRY
400 - MOLECULAR ANALYSIS
500 - MORPHOLOGY
600 - PLATELETS
700 - RED CELL ANALYSIS AND HEMOGLOBINOPATHIES
800 - STANDARDS AND QUALITY ASSURANCE

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NEWHEMATOLOGICAL PARAMETERS IN ASSESSING-SPORT EFFORT

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¹SIMEL CASTELFRANCO VENETO, Italy, ²Laboratorio Patologia Clinica AUSL MODENA, Italy

Introduction: Clinical laboratory main contributions to sports medicine consist in sportmen health state definition, current disease exclusion, workloads evaluation, weaknesses management, illicit behaviours identification and enforcement, avoiding erroneous diagnosis of performance-enhancing drugs abuse. The hematology laboratory provides to doctors and trainers multi-parameter blood test, i.e. fully automated counts and morphological data. More functional informations about leukocytes (WBC) status in athletes of different sport and conditions are now available. In athletes granulocytes increase for a physiological mobilization of normal-mature cells from intravascular marginalized pool and “extravascular storage”. A stress-leukocytosis results, sustained by cortisol changes related to effort adaption. In peripheral blood some dysplastic immature granulocyte, can be found. After physical labor, the blood picture is mostly characterized by neutrophilia, lymphopenia (CD4+), CD4/CD8 ratio imbalance, NK and monococytes small increase, possibly due to temporary deficit of specific immunity, an “open window” of increased susceptibility to some pathogens (3-72h, depending on single immune function). It is still unclear immune reaction “rest/after-exercise” are similar in athletes and others, if demonstrable direct influences on GB exists, if the GB reaction is different in professionals in training and race. Methods: From 2012 and still ongoing, we studied WBC changes in athlete physical activity/different sport effort. Counts, relations between leukocyte clusters, functional and morphological type (mature, immature, dysplastic) were observed performing blood pre-test, post-training-test, post-competition-test (enrolled: professional fin swimmer10, amateur cyclists 22, professionals in training and race. Results: From 2012 and still ongoing, we studied WBC changes in athlete physical activity/different sport effort. Counts, relations between leukocyte clusters, functional and morphological type (mature, immature, dysplastic) were observed performing blood pre-test, post-training-test, post-competition-test (enrolled: professional fin swimmer10, amateur cyclists 22, control group 25) by 3 top hematology analysers and NCCLS H20A review. WBC “function” was evaluated by “cell population” parameters, the latest counter generation data, different analyzer principles linked. Different from traditional morphology but strictly correlated, they indicate hematopoiesis status, as widely validated. Results: According to literature, the obtained results, comparing control group to all athletes, showed increased WBC (till 40%/base value). Neutrophilia, decreased lymphocyte, immature granulocytes absent both in instrumental/microscopic observation; no, atypia detection by microscopic review or analyzer flag. Conclusions: Discussion item are how, when, what instruments/parameters can contribute to the athlete health survey. Hematology laboratory, today, offers some facilities as new parameters, good instrumental performance, automation results standardization, reproducibility and accuracy by highly reliable and non-invasive methods.
CASE STUDY - PROACTIVE MONITORING OF SYSTEM DATA DXH PROSERVICE SOLUTIONS

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Introduction: The UniCel DxH Series of Coulter Cellular Analysis Systems provides Complete Blood Count (CBC), white blood cell (WBC) differential, nucleated red blood cell (NRBC), and reticulocyte analysis. In an environment where turnaround time is critical, physicians require prompt and accurate results and an inoperable system directly impacts the laboratory’s ability to deliver results. Therefore, it is essential to provide a solution that will efficiently analyze and diagnose, in near real time, large amounts of system data for root cause analysis. PROService is a remote management and diagnostics system that facilitates the continuous transfer and analysis of system performance data from Beckman Coulter instruments to Beckman Coulter’s PROService servers. The PROService framework is designed for real time monitoring of instrument system functions and also includes large-scale, multi-dimensional data analysis over time. Incoming data is channeled through PROService applications where the output is used by Beckman Coulter Service, Technical Support and Product Development engineers to proactively diagnose and resolve system issues. Methods: An increase in calls into the Technical Support Center is identified on the DxH 800. A data set representing performance data from over 1800 DxH 800 instruments is collected, collated and analyzed from the instrument subsystems (reagent, hardware, maintenance, software, fluidics) through PROService applications utilizing a Hadoop server to manage, standardize, analyze and graphically present large scale instrument data analysis. The analysis identifies the primary drivers and other related parameters by region over time. Results: A data set of ≈120 million data records was analyzed. Figure 1, identifies graphically, the three key subsystem parameters that correlate to the onset of increased calls and total reports. The data clearly details, by parameter (Y axis), over time (X axis) and by system (red require maintenance; blue are properly maintained) the specific system information used for effective problem identification and resolution. The data also documents that the corrective measures once implemented were effective. Conclusions: The PROService Solution provides real time system monitoring on the DxH series. In addition, the PROService solution includes expanded data analysis and identification capabilities that facilitate large-scale, complex investigations. Several programs working in conjunction enable efficient, large scale data analysis and management to ensure the proactive identification of factors contributing to system down time.

EVALUATION OF SYSMEX XS-1000I HEMATOLOGY ANALYSER FOR DETECTING MALARIA INFECTION IN BRAZIL: PRELIMINARY RESULTS.

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Introduction: 99% of malaria cases in Brazil occur in the Amazon region, mostly caused by P. vivax (82%), and followed by P. falciparum (18%). CBC results provided by some automated hematology analyzers can suggest early diagnosis of malaria. Hemozoin causes a falsely elevated eosinophil count (pseudoeosinophilia) associated with atypical distribution in the WBC scattergram. The Sysmex XS hematology analyzer issues an interpretative message (IP) when the distribution of eosinophils is abnormal. Additionally a flag - pRBC?- is emitted when parasite is present. We have evaluated the performance of Sysmex XS-1000i for detecting malaria infection. Methods: 117 blood samples were analyzed in two laboratories from two different regions in Brazil: 1) 46 samples in the Hospital of the State University of Campinas, São Paulo (Southeast region) - non-endemic area; and 2) 71 samples in the Fundação de Medicina Tropical Dr. Heitor Vieira Dourado, Manaus, Amazonas State (North region) - endemic area. The samples were analyzed on the Sysmex XS-1000i hematology analyzer. Malaria diagnosis was made by microscopy examination of stained thick films (reference method). Results: 42 from 117 samples were positive for P. vivax: Sensitivity of pRBC flag in detecting the infection was 82.3%, specificity 100%, and accuracy 92.2%. The flag sensitivity was low (66.6%) in samples from patients submitted to antimalarial treatment, probably due to the significant reduction of circulating parasites in the first days of treatment. Nevertheless, the specificity of the test was 100% and accuracy was 91.6% in this group of patients. We did not observe pseudoeosinophilia in any of the positive samples. However, abnormalities in WBC scattergram of DIFF channel, such as eosinophilic or neutrophil double populations, and graying of different leukocyte group were found in all positive cases. Conclusions: Sysmex XS-1000i analyzer is useful in detecting P. vivax infection, and a careful analysis of WBC scattergram can be an important tool in the early diagnosis of infection. pRBC flag and abnormalities of WBC scattergram can be the first indication of Plasmodium infection in patients with febrile episodes in non-endemic areas. From these data, specific tests may be required more quickly, speeding up the appropriate therapeutic approach.
104 RAPID EVALUATION OF POST-ANTIBIOTIC EFFECTS BY LEUKOCYTES DIFFERENTIAL SCATTERGRAM
Hiromi Kataoka1, Yasuhiro Ochi2, Yutaka Hatakeyama1, Yoshiyasu Okuhara1, Taisuke Hisahara1, Yoshihisa Matsumura1, Tetsuro Sugihara1
1Center of Medical Information Science, Kochi Medical School Kochi, Japan, 2Sysmex Corporation Kobe, Japan, 3Laboratory Medicine, Kochi Medical School Hospital Kochi, Japan

Introduction: Selection of optimal antibiotics for the treatment of bacterial infection should be based on the microbiological examination results. However, the results are reported several days after. The purpose of this study was to evaluate the therapeutic effect of antibiotic administration based on the variation in leukocytes differential scattergram patterns in the patients with methicillin-resistant Staphylococcus aureus (MRSA) infection.

Methods: Subjects included patients who started their first course of antibiotic therapy within 7 days of MRSA infection and covered for 14 consecutive days. Raw data were obtained using an automated hematology analyzer (Sysmex XE-2100) to measure variations in leukocytes differential scattergram patterns 1 day before and after administration of antibiotic therapy. The raw data were analyzed by unfolding the DIFF channel data to a two-dimensional scatter plot and dividing them into 1,024 (32×32) parts. Difference in pattern variations were compared using receiver operating characteristic (ROC) analysis in 181 patients who had vancomycin (VCM), 33 patients with linezolid, 6 patients with teicoplanin (TEIC), and 58 with sulbac tam/ampicillin (SBT/ABPC).

Results: Scattergram distribution patterns of the treated groups before and after therapy revealed that the number of immature granulocytes from the upper neutrophil region (the square of X axis=18th, Y=21st of 32×32 DIFF channel scatter plot) increased in the VCM, linezolid, and TEIC groups, but not in the SBT/ABPC group. When the ROC analysis was performed by assuming that the VCM administration was effective and that the SBT/ABPC administration was not effective, its area under the curve was 0.71.

Conclusions: The variations of leukocytes differential scattergram patterns were useful to evaluate the effects of MRSA antibiotic therapy early as one day after the administration.
HIGH TITER OF CIRCULATING ANTI-NUCLEAR ANTIBODIES AND VOLUME CONDUCTIVITY SCATTER DATA OF LYMPHOCYTES

Sang-Gyung Kim1, A-Jin Lee2, Hae-Bong Jang1 1Catholic Univ of Daegu Daegu, South Korea, 2Catholic Univ of Daegu Daegu, South Korea.

Introduction: Circulating anti nuclear antibodies (ANA) are diagnostic marker of systemic rheumatic diseases. They are measured by indirect immunofluorescence method or ELISA in clinical laboratories. Complete blood cell count (CBC) using automated blood analyzer is one of the most common routine test in hospital and cell population data (CPD) are easily obtained. We have reported the relationship of procalcitonin and mean volume of neutrophils and monocytes. If it is possible to predict the presence of high titer circulating ANA through CPD and CBC, it is very helpful in clinical practice. Therefore, we investigated the volume conductivity scatter (VCS) parameters of lymphocytes.

Methods: VCS data of 8 patients with any ANA positive with high titer (>1: 320) by IIF (N=8) were analyzed. For control group, VCS data of 15 health examination were used. The VCS data was measured by UniCel Dxi800 Coulter system (BeckmanCoulter, USA). ANA were measured by indirect immunofluorescence methods (immunothink, Seoul, Korea). Mann Whitney test was used for statistical analysis.

Results: The mean conductivity of lymphocytes (MNC-LY) of high titer ANA patients were 124.8±2.1 and 115.2±0.37 of control group. The MNC-LY, and mean upper median angle light scatter (MNUMALS) of high titer ANA patients were significantly higher than control group (p< 0.01). The mean volume of lymphocytes was 87.4±0.74 and 84.4±0.37, respectively. The MNV-LY was not significantly different between the two group. These results suggest that VCS data of lymphocytes, MNC-LY and UMA-LS may be useful to predict the presence of high titer ANA.

SCREENING FOR PAROXYSMAL NOCTURNAL HEMOGLOBINUREA WITH CYTODIFF® ANALYSIS.

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1Russian Medical Academy of Postgraduate Education Moscow, Russia, 2Beckman Coulter Eurocenter Nyon, Switzerland

Introduction: Paroxysmal nocturnal hemoglobinuria (PNH) is a rare hematopoietic stem cell disorder resulting from the somatic mutation of the X-linked phosphatidylinositolglycan complementation Class A (PIG-A) gene. PNH diagnosis can be established by demonstrating the absence of cell membrane GPI-anchored proteins from granulocytes or RBC. It has been also described that the expression of CD16 can be decreased on PNH-affected granulocytes. Recently, a new method for extended flow WBC differential was introduced by Beckman Coulter. This method uses flow cytometric analysis with CytoDiff™ reagent that provides a 10-part WBC differential. This method allows the detection of the abnormal antigen expression on WBC, for example, low CD16 expression on granulocytes. The aim of the study was to evaluate the efficacy of CytoDiff™ analysis of peripheral blood for PNH screening detecting low CD16 expression on granulocytes.

Methods: EDTA-anticoagulated blood samples from patients with PNH suspicion were prospectively included in the study. Analysis of the PNH clones was conducted in accordance with international protocol and ICCS Guidelines. For extended flow WBC differential analysis, the blood samples were stained with the CytoDiff™ panel, lysed with Versalyse (Beckman Coulter) and 20,000 leucocytes were analyzed on a FC500 Flow Cytometer (Beckman Coulter) using CytoDiff™ XP software.

Results: 53 patients with PNH suspicion were analyzed. All patients were characterized by anemia, thrombocytopenia and/or leucopenia. PNH diagnosis was confirmed in 6 patients and in another 7 patients the final diagnosis was aplastic anemia with PNH clone. The remaining 40 patients were not confirmed for PNH. For all 13 patients with confirmed presence of PNH clone, CytoDiff™ reported an increased number of Immature Granulocytes (range 3-45%). Microscopy did not detect the presence of Imm Grans so we were able to conclude that the falsely increased Imm Gran count was due to the decreased CD16 expression on Neutrophils. Good correlation (r=0.93) was observed between IG count and the size of granulocytic PNH clone.

Conclusions: Our data demonstrate that CytoDiff™ analysis provides an efficient screening tool for abnormal CD16 expression on neutrophils. *Not available in the United States and other geographies.
PNEUMOCOCCAL BACTEREMIA REVEALED BY AUTOMATED DIGITAL CELL MORPHOLOGY ANALYSIS

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1Hospital Josep Trueta Girona, Spain, 2Hospital Trias I Pujol Badalona, Spain, 3Hospital Josep Trueta Girona, Spain, 4Hospital Josep Trueta Girona, Spain, 5Hospital Josep Trueta Girona, Spain

Introduction: Automated systems for leukocyte recognition and differential pre-classification such as the CellVision DM96 are currently approved and used in clinical laboratories for morphological leukocytes review. We report a case of pneumococcal bacteraemia from the emergency service that was detected with images captured by CellVision, contributing to improve the response time as well as a correct diagnostic orientation. Methods: A 41 years-old man came to our hospital with dyspnea and general discomfort. He had arthralgias and runny nose. He followed routinely hypertension treatment and underwent splenectomy for benign tumoration. Observations revealed fever (40°C), cyanosis and hypotension (75/55 mmHg). Artery blood gas demonstrated severe acidosis (pH 7.09 and lactate 86 mg/dL). Chest radiography showed no abnormalities. Cell blood count (CBC) was analysed on an automated Sysmex Cell blood count (CBC) was analysed on an automated Sysmex. Among them, we observed intracellular aggregates which seemed to be diplococci (Panel-A). Gram stain processed with digital microscopy showed images that suggested Gram-positive diplococci into neutrophils consistent with intracellular microorganisms. This can lead to an early diagnosis and more accurate treatment of critical patients.

RESULTS: Digital images were analysed after peripheral blood (PB) smear was stained with May-Grümmwald Giemsa and processed in the Cellavision DM96. Among them, we observed intracellular aggregates which seemed to be diplococci (Panel-A). Gram stain processed with digital microscopy showed images that suggested Gram-positive diplococci into neutrophils consistent with widespread bacteraemia (Panel-B). A suspected shock septic due to Gram-positive bacteria was reported. Streptococcus pneumoniae was isolated from the blood culture 24 hours later and the antibiogram revealed penicillin resistance and low minimum inhibitory concentration to 2nd and 3rd generation cephalosporin. Three days after this initial fit the patient suffered multiple organ failure and died. Conclusions: PB smear inspection is essential when an infectious disease is suspected. Automated digital cell morphology is a reliable tool that allows a quickly overall blood morphological analysis and may detect intracellular microorganisms. This can lead to an early diagnosis and more accurate treatment of critical patients.
**Introduction:** The myelodysplastic syndromes (MDS) are a heterogeneous group of clonal diseases in the hematopoietic stem cell, typified by presenting ineffective hematopoiesis, with a bone marrow (BM) normo- or hypercellular with cytopenias in peripheral blood. The diagnosis is based on the discovery of dysplastic traits and blasts in peripheral blood (PB) and/or BM, so as in certain cytogenetic alterations. Dysplasia of the granulocyte series, specifically the hypogranulation, are frequent dysmorphisms in the MDS. The dysplastic traits are unspecific, so they can be presented in other processes, for that reason it is essential to do an exhaustive differential diagnosis. Certain standard parameters provided by the automatic analyzers could constitute a useful tool in the MDS diagnosis.

**Objective:** To study if two structural parameters provided by the Sysmex XE-2100, NEUT-X y NEUT-Y analyzer, can contribute to the MDS diagnosis.

**Methods:** The PB of 39 patients who were diagnosed of MDS and 50 normal controls (NC) were analyzed. The patients with MDS were classified, according to the FAB, with 12 refractory anemia (RA), 11 RA with ringed sideroblasts (RAS), 6 RA with an excess blasts (RAEB), and 9 chronic myelomonocytic leukemia (CMML). The hemograms were processed with the Sysmex XE-2100 (Roche Diagnostics). Parameters about the neutrophils granulation, NEUT-X y NEUT-Y were obtained, and peripheral cytopenias (Hb < 10.0 g/dL, neutrophils < 1800/μL, platelets < 100 x 10^3/μL) were evaluated. The statistical analysis was done through SPSS 19.0.

**Results:** From the 39 MDS studied, 37% presented anemia, 42% neutropenia, 29% thrombocytopenia. 18% out of the patients presented bicytopenia and the 5% pan-cytopenia. It was observed a significant statistical difference at the NEUT-X and NEUT-Y values between the MDS and the NC (NEUT-X: SMD= 1283.61±66.36 vs. NC= 1324.33±52.12; p<0.001, NEUT-Y: SMD= 377.45±49.3 vs. NC= 399.67±19.0; p=0.005). No relation was found between the NEUT-X and NEUT-Y values and the total number of neutrophils, but it did was found a relation with the number of cytopenias (NEUT-X: 0-1 cytopenias=1299.1±50.9 vs. 2-3 cytopenias=1233.67±87.4; p=0.008). The RAEB showed a significant minor value of NEUT-X than the remaining MDS (RAEB: 1221.81±89.7 vs. RA: 1305.5±62.2 (p=0.012), vs. RSA: 1287.27±60.5 (p=0.047) and vs. CMML: 1291.1±42.4 (p=0.047)).

**Conclusions:** The use of hemograms parameters such as NEUT-X, can constitute a useful tool in the initial cytopenia screening and facilitate the MDS diagnosis.
112 BODY FLUID UTILITY IN THE UNICEL DXH 800 ANALYZER: EVALUATION OF APPROVED AND INVESTIGATIONAL PARAMETERS

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Introduction: The UniCel® DxH 800 COULTER® Cellular Analysis System has an automated method for enumeration of the Total Nucleated Cells (TNC) and Red Blood Cells (RBC) in body fluids. It provides the percentages and absolute counts of mononuclear and polymorphonuclear cells as investigational parameters. Moreover, a COULTER Body Fluid Control can be used for control and verification of the measuring range of the TNC and RBC parameters in the body fluid panel. The aim of this study was to compare manual hemocytometry with new UniCel DXH 800 (DXH) in cell counting of body fluids (BF). Methods: During a 8 months period (years 2014 and 2015), we processed several BF sent to the laboratory to cell count by the DxH 800 after the conventional counting by two clinical pathologists with Neubauer camera, and cell identification by means of a Papanicolaou staining, if needed. We used DXH software of body fluids (BL) and total blood (CD) to assess the differences between them. Wilcoxon test for paired samples and test for Pearson’s correlation were used for method comparison.

Results: Eighty-three BF sent to the laboratory to cell count were included, 21 out of which were pleural, 39 peritoneal, 8 cerebrospinal (CSF) and 2 synovial fluids. Only pleural and peritoneal fluids (N=56) could be compared because of too little amount or bad conditions of the rest of the samples. Leukocyte counts by manual and BL-DXH methods were replaceable in all BF, as well as RBC count in peritoneal liquid (r>0, 9 in all cases). Differential counts and percentages for mononuclear and polymorphonuclear cells by the three methods (manual, BL-DXH and CD-DXH) were also equivalent (r=0,.5 for peritoneal, and r=0, 6 for pleural fluids with the BL-DXH method). RBC count methods were not comparable in pleural fluid.

Conclusions: Manual and automated DXH cell methods for absolute counting and WBC differential of pleural and peritoneal fluids were comparable in general, especially with body fluid specific software. DXH body fluid cell count constitutes a valuable, quick tool for laboratories with high workload. Studies with more kinds of samples should be done to confirm these results. Supported by ISCIII, RTICC, FEDER (RD12/0036/0044); 2014 SGR225 (GRE), FIJC and Obra Social “la Caixa”

113 REFERENCE VALUES FOR EXTENDED ERYTHROCYTE, RETICULOCYTE PARAMETERS AND IMMATURE GRANULOCYTES COUNT IN HEALTHY BLOOD DONORS

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Introduction: The importance of local reference values has been well described in the literature since the population characteristics may influence the laboratory tests results. The aim of this study was to establish the reference values for extended erythrocyte (RBC-He, Hypo-He, Hyper-He, Macro-R, and Micro-R), reticulocyte indices (Ret % and absolute values, Ret-He and IRF), and the immature granulocytes (IG) parameters in healthy subjects. Methods: A total of 249 healthy blood donors (125 males and 124 females) from the Blood Bank, Hospital São Lucas, Pontificia Universidade Católica do Rio Grande do Sul, Brazil were enrolled in this study. Blood samples were processed in the hematology analyzer Sysmex XE 5000 (Sysmex, Kobe, Japan). Results: Table 1: Extended erythrocyte reference values.

Table 1: Extended erythrocyte reference values.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Male (n=125)</th>
<th>Female (n=124)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
</tr>
<tr>
<td></td>
<td>RV (CI 95%)</td>
<td>RV (CI 95%)</td>
</tr>
<tr>
<td>RBC-He (pg)</td>
<td>30.35 (12.9)</td>
<td>30.26 (13.4)</td>
</tr>
<tr>
<td>HYPO-He (%)</td>
<td>0.20 (0.10)</td>
<td>0.20 (0.10)</td>
</tr>
<tr>
<td>HYPER-He (%)</td>
<td>1.00 (0.20)</td>
<td>0.90 (0.20)</td>
</tr>
<tr>
<td>MICRO-R (%)</td>
<td>1.40 (1.15)</td>
<td>1.30 (0.98)</td>
</tr>
<tr>
<td>MACRO-R (%)</td>
<td>5.90 (0.95)</td>
<td>5.45 (0.98)</td>
</tr>
<tr>
<td>P value</td>
<td></td>
<td>0.609</td>
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</tbody>
</table>

RV= reference values (CI 95%). SD: standard deviation. *Kolmogorov-Smirnov test (P< 0.05), †Student’s t-test, ‡Mann-Whitney test.

Table 2: Reticulocyte and IG reference values.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Male (n=125)</th>
<th>Female (n=124)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
</tr>
<tr>
<td></td>
<td>RV (CI 95%)</td>
<td>RV (CI 95%)</td>
</tr>
<tr>
<td>RET (%)</td>
<td>1.32 (0.64)</td>
<td>1.36 (0.45)</td>
</tr>
<tr>
<td>RET (μL)</td>
<td>0.07 (0.02)</td>
<td>0.06 (0.02)</td>
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<tr>
<td>RET-He (pg)</td>
<td>33.63 (1.70)</td>
<td>33.18 (1.71)</td>
</tr>
<tr>
<td>IRF (%)</td>
<td>6.62 (3.22)</td>
<td>5.10 (2.78)</td>
</tr>
<tr>
<td>IG (10³/μL)</td>
<td>0.015 (0.014)</td>
<td>0.014 (0.009)</td>
</tr>
<tr>
<td>P value</td>
<td></td>
<td>0.794</td>
</tr>
</tbody>
</table>

RV= reference values (CI 95%). SD: standard deviation. *Kolmogorov-Smirnov test (P< 0.05), †Student’s t-test, ‡Mann-Whitney test.

conclusions: The new erythrocyte and reticulocyte parameters can be useful to analyze further results in different clinical conditions or in patients groups with specific diseases.
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IMPROVED FLAGGING PERFORMANCE OF THE SYSMEX XN2000 HAEMATOLOGY ANALYSER
Margreet Schoorl, Marianne Schoorl, Monique Chevallier, Joost Elout, Hans van Pelt
MCA Alkmaar, Netherlands

Introduction: Hematology analysers generate suspect flags if abnormal cells are present in the samples. With the introduction of the Sysmex XN haematology analyser the white blood cell differentiation channel (WDF) and abnormal cell detection channel (WPC) have been added with improved algorithms for flagging blasts and abnormal or atypical lymphocytes.

Methods: This study evaluated 2011 K2EDTA anticoagulated samples from the daily routine (clinical patients: n=863, outpatients: n=1148). The samples were analysed within 4 hr after collection. For comparison, blood smears were examined with a Cellavision DM96, according to the CLSI protocol H20-A2-2007. The Sysmex XN Haematology analyser was equipped with software version 00.12. Q-flag settings were according to the specifications of the manufacturer.

Results: Based on the Cellavision results the total number of samples on the XN-2000 demonstrated 6 false positive (FP) and 4 true positive (TP) in the blast flag, 6 FP and 9 TP in the abnormal lymph flag, and 15 FP and 0 TP in the atypical lymph flag. Furthermore 9 false negative (FN) were observed in the blast flag, 158 FN in the abnormal lymph flag and 4 FN in the atypical lymph flag. Positive WBC suspect flags were demonstrated in 3% of the samples. In the 58 positive samples the flag Blasts/Abnormal lymph? was demonstrated in 40 cases, the combined flag Blasts/Abnormal lymph? / Atypical Lymph? in 15 cases and the flag Atypical Lymph? in 4 cases. From the 55 samples with the initial WBC IP suspect flags Blasts/Abnormal lymph? or Blasts/Abnormal lymph? / Atypical Lymph? a reflex test was performed within the WPC channel which resulted in a negative flagging in 14 cases.

Conclusions: With the more specific flagging from the WPC channel slide reviews were reduced with 25%. Atypical and abnormal lymphocytes are microscopically difficult to distinguish, which may have resulted in a high number of false-negatives. For proper evaluation additional immunocytological studies should be performed in pathological samples.

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EARLY SUSPICION OF APML: THE PANDA APPROACH USING ADVIA 2120I HEMATOLOGY ANALYSER
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Introduction: Acute Promylocytic Leukemia (APML) is a medical emergency as bleeding is a major cause of early death that demands early recognition and immediate treatment. In many instances, an abnormal complete blood count (CBC) provides the first indication. Modern Sixth Generation Haematology analyzer like ADVIA uses combination of cytochemistry and light scatter measurements to derive the peroxidase activity (PA) and Nuclear Density (ND) to provide information about presence of leukemic cells in peripheral blood.

Methods: A total of 163 cases of AML and 20 CML were assessed retrospectively against the PANDA system. Out of 213 cases, 78 cases were of APML. Out of 78 cases, 64 cases had a PANDA profile compatible with the final diagnosis. Out of 14 cases, 5 cases are negative for t(15;17) and PML-RARA transcript identification and considered as a non-APML AML. Out of 14 cases, 4 cases had WBC count less than 1000/uL, 6 cases had WBC count more than 1000/uL but less than 3000/uL and 4 cases with WBC count more than 4000/uL.

Results: APML is the only AML subtype allocated to P6/D1 PANDA profile and crucially none of the remaining 85 cases of Non-APML AML and 20 CML, displayed this characteristics profile. Therefore, sensitivity was found to be 100 and Specificity is around 81%.

Conclusions: Although PANDA pattern on CBC on ADVIA doesn’t replace conventional diagnostic approaches but it helps in rapid and early suspicion to aid these investigations. It can provide a vital early warning that promylocytic cells may be present in the peripheral blood.
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BODY FLUID ANALYSIS PERFORMANCE EVALUATION ON MINDRAY BC-6800 AUTOMATED HEMATOLOGY ANALYZER  
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Introduction: Body fluids, such as cerebrospinal fluid (CSF), pleural fluid, peritoneal fluid and synovial fluid, are important sample types that can provide valuable diagnostic information for meningitis, encephalopathy, liver cirrhosis and many other diseases. For decades, analysis of body fluid samples depended on microscopic methods, which are labor intensive, inefficient and have poor repeatability. The newly introduced body fluid (BF) mode on Mindray’s BC-6800 can analyze body fluid samples automatically, reporting counts and classification for detected blood cells. This study evaluated the performance of body fluid mode on the BC-6800 analyzer.  

Methods: The body fluid (BF) mode of BC-6800 was evaluated in term of instrument background counts, carryover, precision, linearity and accuracy as compared against microscope based methods in 265 samples that included CSF, pleural fluid and peritoneal fluid specimen.  

Results: The background counts of the analyzer met specification claims of instrument manufacturer viz. WBC-BF≤1×10^6/L RBC-BF≤3×10^9/L). The Carryover study showed highest carryover of 0.07% for WBC-BF and 0.00% for RBC-BF, respectively. Both results are better than the claimed specifications of < 0.3% for both by instrument manufacturer. In instrument precision analysis, the precision for WBC-BF ranged from 0.82%~4.63% while for RBC-BF it was 1.58%~3.46%. Linearity studies showed WBC counts to be linear from 0 to 10000 x10^6/L and RBC to be linear from 0 to 5000 x10^9/L. In WBC accuracy, correlation R^2 of 263 samples between and instrument and microscopic methods was 0.9924 and in RBC accuracy correlation R^2 was found to be 0.9952. Accuracy of PMN# classification correlation R^2 of 142 samples was 0.9944. and for MN# the accuracy correlation R^2 of 142 samples was 0.9944.  

Conclusions: The body fluid (BF) mode on the BC-6800 automated hematologic analyzer can provide accurate RBC and WBC counts with cell differential capability, in clinically significant concentration ranges for body fluid samples. The body fluid (BF) mode on BC-6800 can be effectively used in clinical lab setup.

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THE LUPUS ANTICOAGULANT TEST EXPERIENCE IN 1429 PATIENTS ATTENDING INFERTILITY CLINIC  
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Introduction: Routine investigation for recurrent pregnancy loss includes measurement of antiphospholipid antibodies under the perception that the lupus anticoagulant (LAC) is prevalent in this population. Our infertility clinic sees 360 patients with recurrent pregnancy loss annually, in addition to those with systemic lupus erythematosus and/or antiphospholipid syndrome.  

Methods: We investigated presence of LAC in patients attending infertility clinic with possibility of high-risk pregnancy. LAC was assessed by two different methods using the following assays: PTT-LA (Lupus Anticoagulant-Sensitive APTT Reagent) and dRVVT (STA-Staclot® dRVVScreen), as recommended by the International Society on Thrombosis and Haemostatis (ISTH) guidelines.  

Results: Of 1429 patients tested for LAC during a 5-year period, 439 (30.7%) tested screen positive on sensitive aPTT test with silica activator & low phospholipid. PTT LA confirmation test was done in tested in 384 screen positive patients and only 8 (2.1%) patients was positive. In dRVV integrated tests with factor 5 activation, 16 (1.1%) patients among 1429 was screen positive. And 14 (1.0%) patients among 1429 was confirmed as presenting LAC. Only 2 patients was false screen positive in dRVV integrated test revealing high predictive value.  

Conclusions: LAC was absent in an overwhelming majority of women with exclusively early recurrent pregnancy loss but was associated with sporadic stillbirth. We found that a confirmed LAC was very infrequent even in a high-risk group.
Introduction: Accurate measurement of FXIII activity is important for the diagnosis and management of patients with FXIII deficiency. In most commercial assays, determination of FXIII activity is based on measurement of ammonia released during the transglutaminase reaction. Reactions generating FXIII-independent ammonia production can lead to over-estimation of FXIII activity by a few percent, which could be clinically significant in samples with low activity. Use of a plasma blank is recommended to correct for these reactions. We report on the validation of a commercial FXIII activity assay on a coagulation analyzer, including automation of a plasma blank measurement.

Methods: We validated the performance characteristics of a commercial FXIII activity method (Technochrom Factor XIII, Technoclone, Austria) on a BCS coagulation analyzer (Siemens Healthcare, USA). Results were expressed as % of normal activity. A plasma blank was measured on samples with < 30% FXIII activity, using a separate calibration curve, and used as a correction factor. Results: 31 plasma samples with a range of FXIII activity were tested by our automated Technochrom method and compared to results from a reference laboratory. 12 samples demonstrated FXIII activity < 30% and were re-tested and corrected for results from a plasma blank. Overall, good correlation was observed between the 2 laboratories (R = 0.98). For the 19 samples with activity >30%, mean values were 91% and 81% for our method and the reference laboratory, respectively. For the 12 samples with low activity, the mean activities in the 2 laboratories were 9% (corrected) and 17%, with higher values from the reference lab in 11 of 12 samples, suggesting that a plasma blank was not performed at the reference laboratory. The average plasma blank value was 6%. Between-run precision studies demonstrated coefficients of variation of < 10% in samples with normal, borderline abnormal, and low FXIII activity. Conclusions: To our knowledge, we are the first laboratory to automate a FXIII activity assay that utilizes a plasma blank. Our data confirm that measurement of a plasma blank is important to avoid overestimation of FXIII activity in deficient samples.
203  SPANISH EXTERNAL QUALITY ASSESSMENT SCHEME IN HEMOSTASIS. QUALITY SPECIFICATION COMPLIANCE.
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Introduction: In 1999, the Stockholm International Conference established a hierarchy of models to set up global analytical quality specifications in Laboratory Medicine in which the evaluation of the effect of analytical performance on clinical decisions, according to biological variation (BV) and the recommendations from expert local groups, were included as second and third position respectively. Objectives: The objective of this work is to establish the degree of compliance with three published quality specifications (QS) for total error (TE) of the results obtained by different coagulation analytical systems in an External Quality Assessment Scheme (EQA-H). Methods: In 2014, Spanish EQAS-H sent a total of 24 plasma samples (12 rounds) to 531 laboratories controlling 645 coagulation analyzers. The target values for TE calculation were the trimmed peer group consensus mean. The peer groups were constituted as a combination of analyzer and reagent. A mean of 12670 results of Prothrombin Time (PT), Partial Thromboplastin Time (PTT) and fibrinogen (Fib) were analyzed. Desirable (d) and minimum (m) BV database for Total Error (1) and Spanish consensus minimum analytical quality specifications (MQS) published by a Spanish Group of Experts (2) were used. We calculated the percentage of results within the three QS by each group of methods. Results: Over 90% of results meet the MQS criterion for the three studied magnitudes. The between groups accepted results according dVB ranged: PT: from 46.3% (IL ACL + IL PT Fib HS) to 71.6% (Stago Neoplatin Plus); PTT: from 51.8% (Stago STA APTT) to 79.6% (IL Hemosyl Synthesil); Fib: from 74.7% (Siemens Thromborel) to 90.3 (Stago STA Fib Reagent). Conclusions: 1) dVB is arduous to meet for coagulation parameters and the use of a less restricted criterion as mVB should be considered. 2) MQS are easily complied by all method groups. References: (1)www.secu.es/es/Sociedad/Base_de_datos_de_Variacion_biologica (2) Ricós C, Ramón F, Salas A, Buño A, Calafell R, Morancho J, Gutiérrez G, Jou JM. Minimum analytical quality specifications of inter-laboratory comparisons: agreement among Spanish EQAP organizers. Clin Chem Lab Med. 2012;50:455-461.

204  PROVISION OF EXTERNAL QUALITY ASSURANCE FOR POINT OF CARE TESTING IN HEMOSTASIS
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Introduction: Point of care (POC) testing for haemostasis tests has increased over recent years in both number of tests being performed and in the tests available in a POC setting. POC tests require quality control and it is the aim of UK National External Quality Assessment Scheme for Blood Coagulation (UK NEQAS BC) to provide suitable External Quality Assurance (EQA) that is tailored to meet the needs of the POC staff. Participation is not restricted to UK centres and currently 11 different countries are represented. Methods: A review of the programme for POC testing offered by UK NEQAS BC was undertaken and results presented here Results: Currently UK NEQAS BC provides POC EQA programmes for INR monitoring, ACT testing, thromboelastography, thrombelastometry and D-dimer testing. The type of test often determines the placement and type of staff performing the test. For example the majority of POC INR monitoring (75%) is performed in primary care by nurses, whereas the majority of thromboelastography/thrombelastometry is performed in secondary care in theatres by anaesthetists or perfusionists. In the last 10 years the UK NEQAS POCT INR programme has seen a 7 fold increase in participation, from 620 in 2005 to 4330 in 2015. UK NEQAS BC has also established POC test programmes for ACT (n=97 users), D-Dimer testing (n=116 users), and thromboelastography/thrombelastometry (n=57 users). The instructions to users and reports have been tailored to make them easily understood by non laboratory staff and the introduction of photo instructions to new users has proved effective in introducing these staff to EQA testing. Regular questionnaires to this group have provided useful feedback in order to develop the programme further Conclusions: Development of the UK NEQAS BC programmes has led to increases in both the number of participants and the number and variety of tests offered. The aim of the programmes is to support and educate centres involved in all aspects of haemostasis testing and improve patient testing by providing EQA suited to users’ needs.
Introduction: Laboratory determination of fibrinogen/fibrin degradation products (FDP) levels is important as hyperfibrinolytic state marker in patients with disseminated intravascular coagulation or primary hyperfibrinolysis, and also post fibrinolytic therapy. The aim of the study was to verify the analytical performance of an immunoturbidimetric (IMMT) assay for FDP [HemosIL FDP, Instrumentation Laboratory] and it comparison to a latex agglutination semiquantitative (SCLAT) test (FDP Plasma, Diagnostics STAGO).

Methods: IMMT was performed on an automated coagulometer ACL TOP 500 (Instrumentation Laboratory) and SCLAT by hand technique. Total accuracy was calculated following the protocol EP 15 at two levels of controls (IL). For linearity protocol EP6 was performed by using 6 different dilutions of one calibrator or a patients’ plasma with similar FDP levels. EP evaluator program was used. For methods comparison 38 consecutive samples from 34 different patients were used. Concordance between methods results in terms of positive and negative, and in categories, <10, 10-20, 20-40, 40-80, 80-160, >160 mg/mL, was calculated through calculated through kappa coefficient (k) in IBM SPSS program. Results: We observed that Total CV% met manufacturer claimed value (within 95% CI) at both levels of controls. Mean values: low control 10.8 mg/mL and high control 31.3 mg/mL. Total CV%: low control 6.0 vs. 4.8 and high control 2.7 vs. 3.6, calculated vs. manufacturer claimed, respectively. Linearity calculated by using calibrator (125 and 3.8 mg/mL) and that calculated by using a patient’ sample (123 and 3.8 mg/mL), showed polynomial fit analysis applicable. When comparing IMMT to SCLAT, a very good concordance was observed between them: k of results expressed as positive–negative = 0.908 (p < 0.001), k of results expressed as categories = 0.817 (p < 0.001). There were only three values that fall in different categories but closest, and only one sample gave low positive (> 10 < 20 mg/mL) and negative (< 10 mg/mL) results, for SCLAT and IMMT, respectively. Conclusions: The analytical performance of the IMMT was acceptable, and the concordance with SCLAT observed by patients’ samples evaluation was very good. The implementation of IMMT in the clinical laboratory is suitable and reliable with the potential advantage that it could be tested in the same coagulometer were coagulation tests are performed simultaneously.

Table 1. Sample results of dRVVT and SCLAT screen and confirm tests. dRVVT S: dRVVT screen, dRVVT C: dRVVT confirm, dRVVT TR: dRVVT Time ratio, SCT S: silica clotting time screen, SCT C: silica clotting time confirm, SCT TR: silica clotting time ratio. P: Positive, N: Negative.
207 PRECISION AND THROUGHPUT OF LIGHT TRANSMISSION AGGREGOMETERY ON A ROUTINE COAGULATION ANALYSER
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Introduction: For the last 50 years, the investigation of platelet function disorders by light transmission aggregation has been a labour intensive process using manually operated instrumentation. The potential to automate this testing on a routine coagulation analyser has recently been demonstrated. Using normal samples, we looked to determine the appropriateness of automated platelet aggregation on a routine coagulation analyser. Methods: We assessed the throughput and imprecision of performing platelet aggregation studies on platelet rich plasma (PRP) on both the CS-2000i and CS-5100 (Sysmex Corporation, Japan). Throughtput was determined using different combinations of samples, agonists and concentrations. We looked at the imprecision of the aggregation response in two samples using five agonists at a single concentration; (ADP and Epinephrine 5mM; Collagen 4mg/ml; Ristocetin 1.0mg/ml and Arachidonate 1.0mM). All agonists were obtained from Hyphen Biomed. Imprecision studies were performed by running each sample five times simultaneously on both the CS-2000i and CS-5100. Finally, we looked at the ability to perform platelet aggregation in response to non-standard platelet agonists (e.g. TRAP peptides, U-46619 and calcium ionophore A-23187 [Sigma Aldrich]). Results: Platelet aggregation on the automated analysers was found to be rapid, with an aggregation run comprising a single sample tested with three agonists with five concentrations of each (i.e. 15 responses) taking 26 minutes to complete on the CS-2000i, compared with 15 minutes on the CS-5100. A sample run comprising five agonists at a single concentration took 16 minutes to complete on the CS-2000i compared with ten minutes on the CS-5100. A run comprising three PRP samples tested with five agonists each at two different concentrations (except Arachidonate at a single dose) took 46 minutes to complete on the CS-2000i, compared with 28 minutes on the CS-5100. Acceptable precision was observed for maximum aggregation (MA) with both analysers for all agonists and concentrations used (CV range - CS-2000i: MA 2.6 – 9.0%, CS-5100: MA 1.4 – 6.8%). Aggregation was comparable to traditional turbidometric methods when U46619, TRAP and A-23187 were used. Conclusions: Our studies demonstrate that automated light transmission aggregometry can now be performed with a fast turnaround time and acceptable precision on a routine, random access coagulation analyser using a wide variety of agonists.

208 VARIABILITY IN EXPOSURE OF EPITOPE G40-R43 OF DOMAIN I IN MANUAL AND AUTOMATED COMMERCIAL ANTI-BETA2GLYCOPROTEIN I IGG IMMUNOASSAYS
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Introduction: A major problem for diagnosing the antiphospholipid syndrome (APS) is the high variability between commercial anti-beta2glycoprotein I (β2GPI) assays. Anti-β2GPI antibodies constitute a heterogeneous population, but predominantly antibodies reacting to the cryptic epitope Glycine40-Arginine43 (G40-R43) in domain I of β2GPI are associated with thrombosis. Coatings of β2GPI may differ in exposure of this pathogenic epitope and influence the performance of the assays. Methods: Two patient-derived monoclonal antibodies were tested on neutral versus anionic plates to test affinity towards domain I of β2GPI. Antibody P1-117 reacts with epitope G40-R43 in the open conformation while P2-6 recognizes β2GPI irrespective of its conformation. These antibodies were tested in manual (A-E) and automated (G-H) commercial anti-β2GPI IgG assays. Furthermore, 196 patients were tested with 2 manual assays (A and B) to investigate the effect on APS diagnosis. Results: In assay A and G, both antibodies showed equal reactivity towards β2GPI, indicating that all the β2GPI exposes G40-R43. In all other assays P1-117 displayed lower reactivity than P2-6, demonstrating reduced G40-R43 availability. To exclude influences of other assay features, reactivity was re-examined on plates of assay A and B using the protocol/reagents from each assay. In all combinations, reactivity of both antibodies was comparable to results obtained with the protocol/reagents of assay A/B, suggesting that coating accounts for the observed differences. In two independent patient cohorts we demonstrated that a number of domain I-reactive samples are missed in manual assays characterized by a reduced exposure of epitope G40-R43. Conclusions: Exposure of epitope G40-R43 on β2GPI is highly variable in manual and automated commercial anti-β2GPI IgG assays. As a consequence, patients can be falsely assigned negative in assays characterized by a reduced exposure of G40-R43. Antibodies P2-6 and P1-117 may serve as controls to ensure sufficient exposure of epitope G40-R43.
209 INCREASED LEVELS OF ACUTE PHASE REACTANTS IN NIGERIANS POST-DIALYZED PATIENTS AS RISK FACTORS FOR THROMBOSIS.
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Introduction: Worsening rheologic profile has been associated with azotaemic patients undergoing haemodialysis. Also, Fibrinogen, an acute phase reactant protein has been reported to increase after dialysis but records of the implication of acute rise in C-reactive protein (CRP) – a specific inflammatory marker as well as plasma Fibrinogen concentration (PFC) at the immediate post dialyzation are scanty. We aimed therefore to identify these parameters as thrombotic risk factors immediately after dialysis.

Methods: A total of 32 Azotaemic patients undergoing dialysis at Ahmadu Bello Teaching hospital, Zaria, Nigeria were studied. Blood samples were obtained pre and post dialysis (1hr, respectively) for the analysis of PFC, CRP, Whole blood and Plasma viscosities (WBV and RPV, respectively) using standard laboratory and statistical methods. A written ethical permit was obtained from the Hospital Research and Ethical Committee before the commencement of the study.

Results: We recorded a significantly higher values of CRP and PFC as well as PV in the 1hr post dialysis samples (P < 0.05, respectively) while WBV showed an observable increase but not statistically significant.

Conclusions: Significantly raised levels of PFC coupled with that of CRP could be adduced to post-dialysis inflammatory responses as positive risk factors of thrombosis immediately after dialysis. This underscore the absolute need for greater post-dialysis care especially at the first few hours as well as the inclusion of these parameters in dialysis patient management.

300 ANALYSIS OF NORMAL AND LEUKEMIC STEM CELLS USING MULTIPARAMETRIC FLOW CYTOMETRY.
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Introduction: In the bone marrow of patients treated for acute myeloid leukemia (AML), the detection of minimal residual disease is a fundamental point to address for better therapy regimens. It has been shown that MRD could be detected in more than 80% of the cases by multiparameter flow cytometry (MFC) at a \textsuperscript{-4} sensitivity threshold using specific combinations of monoclonal antibodies (MoAb) and leukemia associated immunophenotypes (LAIP). Nevertheless, immunophenotype modulations are frequent during chemotherapy and prevent in many cases a good follow-up of MRD. Another alternative is to compare normal and leukemic bone marrow immunophenotypes in order to detect abnormal cells. This process seems very promising and was previously reported (Allou K et al, ISLH 2013). Recently, differences between normal and leukemic stem cell immunophenotypes were reported and used for MRD follow-up. We applied an MFC procedure for the detection of leukemic stem cells directly compared to their normal stem cell counterpart.

Methods: Fifteen normal bone marrow samples obtained from a surgery department were compared to 20 samples of AML at diagnosis and follow-up using MFC procedures. The MoAb panel was chosen as follows:

CD44+/CD38- cells were proven to contain both normal and leukemic stem cells. Comparisons between normal and abnormal samples were performed using a specific gating strategy on Kaluza™software (Beckman Coulter).

Results: Using adapted Kaluza™gating and analysis (in particular, the radar procedure), it was possible to individualize normal and abnormal stem cells at diagnosis and follow-up for every patient, with the three combinations of the panel.

Conclusions: A precise distinction between normal and leukemic stem cells can now be reached introducing a new panel of monoclonal antibodies and new software analysis. Therefore, a better detection of MRD in AML is suggested by these preliminary results and should be confirmed on larger series of patients.
301 MULTIPARAMETRIC (10 COLOURS) FLOW CYTOMETRY OPTIMISATION FOR IMMUNOPHENOTYPING OF HAE-MATOLOGICAL MALIGNANCIES
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Introduction: Multiparametric flow cytometry immunophenotyping has become an indispensable tool in the diagnosis of haematological malignancies. In this study, we developed a in house validation protocol for our ten colour panel in Navios, Beckman Coulter (BC) as currently available guidelines have limited information on specific reagent cocktails, instrument setups and analysis strategies. Methods: Figure 1 illustrates our antibody-flourochromes and clones selected for our leukaemia and lymphoma panel. A systematic evaluation was carried out with assessment of lysing solutions, fixatives, antibodies and correlation of the findings with our current eight colour panel on Facs Canto II (Beckton Dickinson, BD). Instrument settings for scatter properties (FSC, SSC) and photo multiplier tubes (PMT) half peak CV were established by running 30 unstained normal blood samples. These settings were applied to FlowSet Pro Flourophores (BC) for daily monitoring of instrument stability. We then evaluated antibody-flourochromes and clones for our custom combination of ~ 80 antibodies from various manufacturers. Antibody titrations were performed and optimal concentrations were determined by assessing the Mean Flourescence Intensity (MFI) and Signal to Noise Ratio (S: N).

Next, label specific compensation setup was performed using COMPtrol Kit (Spherotech) and verified by running 100 cases in parallel with our current eight color panel. We also prepared antibody cocktails for our 10 colour panel which was verified by daily normal stained blood quality control monitoring. Results: Our findings indicated that optimization of various clones and antibody-flourochromes from different manufacturers can be done with extensive and rigorous validation. An open system selection of antibody fluorochromes can be adapted into any flow cytometer by using the right filters to suit the fluorophores. We also evaluated the newly introduced Brilliant Violet fluorophores for the violet laser (405nm). Filter configurations were modified for BV510 in our instrument. Outstanding performance of BV (Biolegend) and Krome Orange (BC) were observed in the violet channels. Our findings also showed that label specific compensation is critical especially in using tandem dyes. Besides that, our daily quality control on a using antibody cocktails proved that tandem dyes are stable when prepared as a multicolour cocktail for approximately 2 months with proper handling. Conclusions: Multiparametric flow cytometry immunophenotyping is the way to move forward in improving diagnosis of haematological malignancies. To our knowledge, this is the first validation and optimization of a 10 colour panel in a clinical laboratory setting in South East Asia.

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APPLICABILITY OF A TEN-COLOR SCREENING TUBE FOR HEMATOLOGIC MALIGNANCIES IN BRAZILIAN CLINICAL LABORATORY
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Introduction: Flow cytometry (FC) has become an essential tool for diagnosis and monitoring of hematological diseases. It has been possible to develop a multiparametric FC in clinical laboratory. As a result of the partnership between the Laboratory Medicine Program of University Health Network, Canada, and the Hospital Israelita Albert Einstein, São Paulo, here in Brazil, we have introduced a screening tube (ST) (10-colors, 14-fluorochrome-conjugated antibodies and 17-parameters) for screening hematologic malignancies in our FC routine. In this study, we discuss the applicability of 10-color tube for screening of hematologic malignancies, and mainly the evaluation of dysplasia using score systems such as Ogata and some criteria of Wells. Methods: This prospective study included 102 bone marrow (BM) samples with heterogeneous clinical hypothesis (CH). The samples were stained with a cocktail of antibodies (CD4FITC+smKappaFITC/CD8PE+smLambdaPE/CD3ECD+CD14ECD/CD33PE-Cy5.5/CD20PE-Cy7+CD56PE-Cy7/CD34APC/CD19APC-A700/CD10APC-A750/CD5PB/CD45PO), analyzed by Navios Flow Cytometer and Kaluza Software (Beckman Coulter). Results: Before the ST phenotypic study, all the samples were evaluated according to the CH and cytomorfolgy (CM). We evaluated the capacity of the ST to finalize the phenotype study, and 24, 5% (25/102) samples did not require complementary tubes. Interestingly, in 5, 9% (6/102) samples, the tube could also establish B-cell clonality. In 53% (54/102) samples the FC study was expanded. In 16, 6% (17/102) samples the FC could detect Ogata score ≥3. Among these samples, 64, 7% (11/17) had CM and FC results suggestive of MDS, and 35, 3% (6/17) showed dysplastic features only by FC, and this CH were related to myeloproliferative neoplasms and pancytopenia. Our results showed good correlation between FC and CM, especially to negatively predict dysplastic features in MDS investigation. In cases that the Ogata score was ≥3, the FC study was, mandatorily, expanded in order to evaluate the granulocyte maturation, monocyte according to some criteria of Wells score, and erythrocytes lineage by CD36 and CD71 expression. The screening tube was capable of detecting the majority of the BM cell populations, including aberrant antigen expression and clonality status on B-cells. Although this tube is capable of evaluating T-cells, no abnormal T-cell population was detected. Conclusions: Use of 10-color screening tube improved our assessment of MDS features. It was possible to detect abnormal expression, aberrant markers and evaluate maturation of B and myeloid cells using fewer samples, with significant reduction in time and increase the sensitivity to aid MDS diagnosis.

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FREQUENCY OF FINDING GPI-DEFICIENT CELLS UNDER VARIOUS CONDITIONS: A REVIEW OF 982 SEQUENTIAL TESTS
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Introduction: An international consensus-based technique for high resolution testing of GPI-deficient cells (GPI-testing) and indications for such testing has recently been described. We report the frequency of occurrence of GPI-deficient cells under different indications with a view to rationalize the low-yield indications. Methods: We reviewed the results of 982 sequential GPI-tests using high resolution flow cytometry, on 873 patients over a 3.5-year period. A 7-color panel comprising of CD45, CD15, CD33, CD64, CD14, CD24 and FLAER was used to assess neutrophils (gated with CD15) and monocytes (gated with CD33 and CD64), while a 2-color panel consisting of CD235 and CD59 was used for RBCs (gated with CD235). Indications for testing were recorded from the requisition and verified by reviews of clinical chart, complete blood count, bone marrow examination, cytogenetics, and laboratory tests for hemolysis including hemoglobin, reticulocyte count, lactate dehydrogenase, bilirubin, haptoglobin, plasma hemoglobin, hemoglobinuria and hemosiderinuria. Results: A positive result (i.e. presence of GPI-deficient cells) was seen in 94/982 (9.6%) tests in 42/873 (4.8%) patients of the clone size of GPI-deficient cells varied from 0.01 to 98%. The frequency of finding GPI-deficient cells was highest in cases of known PNH (39/45, 86.7%) and lowest in unicytopenia (1/226, 0.4%). Of the cytopoena cases, the GPI-deficient cells were detected most frequently in cases with pancytopenia (15/151, 9.9%) and least frequently in unicytopenia (1/226, 0.4%). Of the cases with unicytopenia, those with anemia were more likely to have GPI-deficient cells (1/145, 0.7%) compared to leukopenia (0/42) or thrombocytopenia (0/39). Conclusions: Using high resolution GPI-testing, frequency of finding GPI-deficient cells varied from a low of 0.4% in cases with unicytopenia to a high of 86.7% in cases with known PNH. The subcategory of unicytopenia as an indication for GPI-testing may be revised in light of the low yield of finding GPI-deficient cells.

<table>
<thead>
<tr>
<th>Frequency of Finding GPI-Deficient Cells in Various Indications</th>
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<tr>
<td><strong>Indication for Testing</strong></td>
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<tr>
<td>Known PNH</td>
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<tr>
<td>Aplastic Anemia</td>
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<tr>
<td>Cytopenia</td>
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<tr>
<td>Pancytopenia</td>
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<td>Hemolytic</td>
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<td>Thrombosis</td>
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<tr>
<td>Myelodysplastic Syndrome</td>
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<td>Others or Not Stated</td>
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MEMORY T CELL RECONSTITUTION AFTER HAPLOIDENTICAL HEMATOPOIETIC STEM CELL TRANSPLANTATION USING TCRαβ-DEPLETED GRAFTS IN PEDIATRIC PATIENTS
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Introduction: Haploidentical hematopoietic stem cell transplantation (HHSCT) with TCRαβ-depleted graft is associated with good prognosis. There are only few studies about memory T cell reconstitution after HHSCT. In this study, we performed flow cytometry-based TCRαβ and TCRγδ T cell subpopulation analysis to observe the patterns of memory T cell reconstitution after TCRαβ-depleted graft-based HHSCT. Methods: Total 27 pediatric patients were enrolled. Diagnoses were hematologic malignancies (HM) and aplastic anemia (AA). 1 patient was experienced graft failure and 3 patients were expired. Peripheral blood samples were regularly collected from infusion day (PID0). 10-color flow cytometric analysis using anti-CD45, CD3, CD4, CD8, CD27, CD28, CD45RA, CD45RO, CD62L, and TCRγδ antibody were performed to identify memory T cell subpopulations [naïve, central memory (CM), and effector memory (EM)]. Results: Among the CD4+ lymphocytes, the percentage of CM was highest until PID 180 (CM 33.8%, EM 13.7%, naïve 19.1%). After PID 181, naïve CD4+ T cell was highest (CM 34.4%, EM 22.0%, naïve 65.6%). This trend was similar in CD8+ lymphocytes. Among TCRγδ lymphocytes, the percentage of CM was consistently highest during overall period (CM 48.3%, EM 9.5%, naïve 37.0%). The proportion of TCRγδ CM after PID365 were significantly higher in HM patient group (39.3%) than AA patient group (12.7%). Until PID 28, all proportions of CD4+ and CD8+ memory T cells until PID 28 were significantly higher in survived patients (P<0.01). After PID 28, the proportion of TCRγδ naïve T cell was significantly higher in survived patients (P=0.043). There was no significant differences of memory T cell reconstitution between graft failure patients and engraftment patients. Conclusions: After TCRαβ-depleted graft-based HHSCT, memory T cell subpopulation showed significant changes. Immediately after HHSCT, CD4 and CD8 memory T cells showed significance in survival. Thereafter, TCRγδ naïve T cell showed significance in survival. There were no significant relationships among memory T cell subpopulation and graft failure. More studies of the dynamics of memory T cell reconstitutions after TCRαβ-depleted graft-based HHSCT is needed.

COMPARISON OF NEUTROPHIL AND MONOCYTE CLONE SIZE IN PATIENTS WITH GPI-DEFICIENT CELLS
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Introduction: Neutrophils and RBCs are routinely tested for expression of glycosyl phosphatidyl inositol-associated antigens (GPI-testing) in paroxysmal nocturnal hemoglobinuria (PNH) and certain bone marrow failure states. However, monocytes are not routinely tested, and their clinical utility is unknown. We report here comparison of results of GPI-testing in neutrophils and monocytes using high resolution flow cytometry. Methods: High resolution GPI-testing was done in 982 instances on 873 patients over a 3.5 years period. A 7-color panel consisting of CD45, CD15, CD33, CD64, CD14, CD24 and FLAER was used for assessment of neutrophils (gated with CD15) and monocytes (gated with CD33 and CD64), while a 2-color panel consisting of CD235 and CD59 was used for RBCs (gated with CD235). Minimum 25 GPI-deficient cells making a tight cluster in two parameter dot plot were deemed necessary to consider a specimen positive. Results: A positive result (i.e. presence of GPI-deficient cells) was seen in 94/982 (9.6%) tests in 42/873 (4.8%) of patients. The clone size of GPI-deficient cells varied from 0.01 to 98.00%. All the patients with positive GPI-testing in neutrophils were also positive in monocytes, and vice versa. In 56/94 positive results, the size of the monocyte clone was higher than neutrophils. Variance between the clone size of the neutrophils and monocytes ranged from -48% to +32%. The clone size is a soft criterion for the diagnosis of PNH, and is also a determinant for prophylactic anticoagulation. In 11 cases, the clone size of neutrophils and monocytes varied by >10%. Of these, two cases of PNH had a considerably larger monocyte clone that would have been clinically significant. Conclusions: Qualitative (positive or negative) results for GPI-testing for neutrophils and monocytes are generally synchronous. However, quantitative (clone size) results for the two cell types vary widely, and the difference may be clinically significant.
307 VALUE OF QUANTITATIVE ASSESSMENT OF MYELOID NUCLEAR DIFFERENTIATION ANTIGEN AND OTHER FLOW CYTOMETRIC PARAMETERS IN LOW GRADE MELODYSPlastic SYNDROME
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Introduction: Diagnosis of myelodysplastic syndrome (MDS) based on morphology is particularly difficult in low grade (LG) MDS (i.e. < 5% blasts, no ringed sideroblasts and karyotypic abnormality). Hence, a more objective method is essential. Thus, the role of myeloid nuclear differentiation antigen (MNDA) and other flow cytometric (FCM) parameters in the diagnosis of LG-MDS was evaluated. Methods: Bone marrow aspirates (BMA) collected from 34 consecutive patients with suspected clinical diagnosis of MDS were divided into three groups: (1) proven MDS (n=12) based on morphology and/or cytogenetics (2) suspected MDS (n=6), non-contributory morphology and cytogenetics but with unexplained cytopenias and (3) non-MDS (n=16). Eleven control BMA were also studied. All cases were analyzed for MNDA expression (on granulocytes, blasts, monocytes and lymphocytes) and seven quantitative parameters: (1) CD34+ myeloblasts % in nucleated cells (2) CD34+ B-cell progenitor % in all CD34+ cells (3) lymphocyte/myeloblast CD45 mean fluorescence intensity ratio (4) granulocyte/lymphocyte SSC peak channel ratio and the proportion of CD34+ myeloblasts expressing (5) CD15 (6) CD11b and (7) CD56. Receiver operating characteristics (ROC) curves were constructed for each parameter. For quantitative assessment, a score of 1 was given to each of these parameters beyond the cut-off and score >3 was considered as FCM positive. Results: MNDA expression on granulocytes and blasts was significantly low in proven MDS vs non-MDS (p=0.0018 and 0.0003, respectively). ROC obtained MNDA cut-off for granulocytes (70.8%) and blasts (81.6%) discriminated suspected MDS from non-MDS when both were altered (p=0.022) but not when only one of these was abnormal (p=0.336). There was no significant difference in MNDA expression on monocytes between MDS and non-MDS cases. In other quantitative FCM parameters assessed, a score of >3 successfully distinguished suspected MDS from non-MDS (p=0.028) with a sensitivity and specificity of 83.3% and 92.6% respectively. MNDA and FCM score, together helped in detection of one suspected MDS case (1/6, 16.7%) missed by the latter alone. However, it mis-categorized one control (1/11, 9.1%). Conclusions: MNDA expression is an independent marker for the evaluation of dyspoiesis and may be added to the standard panel for quantitative assessment by FCM.

308 MEASUREMENT OF LYMPHOCYTE AGGREGATION BY FLOW CYTOMETRY-PHYSIOLOGICAL IMPLICATIONS IN CHRONIC LYMPHOCYTIC LEUKEMIA

Introduction: Cellular aggregation is a physiological response of lymphocytes to various extracellular stimuli. Currently, lymphocytes aggregation is only evaluated qualitatively or by semi-quantitative methods. In the present study we assessed the capacity of flow cytometry to measure lymphocytes aggregation in a quantitative, accurate and reproducible manner, and examined the significance of aggregation responses in various lymphoproliferative diseases. Methods: Extracellular stimuli were utilized to trigger lymphoid aggregation in a concentration dependent manner. Aggregation was quantified by flow cytometry based on the forward or side scatter, or by dark-field side scatter of aggregates measured by ImageStreamX. Accuracy, reproducibility and limitations of the methodology were evaluated. Aggregation responses were measured in various types of lymphoproliferative diseases, and correlated with immunophenotyping and IGHV mutational status in chronic lymphocytic leukemia. Results: Extracellular stimuli such as anti-CD19 antibodies or phorbol ester provoke lymphoid aggregation that can be measured by flow cytometry, in a concentration dependent manner. Once formed, lymphoid aggregates exhibit elevated forward and side scatter signals relatively to their cellular content. Side scatter measurements are linear with the cell numbers contained in each event up to 6 cellsaggregate. Side scatter can also measure aggregates containing more than 6 aggregates, but these cannot be resolved accurately. In contrast, the link between forward scatter and cellular aggregation is not linear, but provides the best resolution between single cells and doublets. Aggregation responses vary among different types of lymphoproliferative diseases. Moreover, elevated levels of CD19-induced aggregation are associated with aberrant chronic lymphocytic leukemia characteristics, but not with IGHV mutational status of the disease. Conclusions: We have demonstrated that flow cytometry can provide accurate and reproducible measurement of both primary chronic lymphocytic leukemia cells, as well as T and B cell lines aggregation in response to physiological stimuli. The use of quantitative evaluation of activation driven or other cellular aggregation may provide an analytical tool to elucidate biochemical and molecular mechanisms associated with lymphoproliferative diseases.
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GENOMIC ANALYSIS USING CUSTOM COMBINATION OF ARRAY COMPARATIVE GENOMIC HYBRIDIZATION (CGH) AND SINGLE NUCLEOTIDE POLYMORPHISM (SNP) ARRAY IN ACUTE MYELOID LEUKAEMIA
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Introduction: Genome wide analysis using array comparative genomic hybridization (CGH) and single nucleotide polymorphism (SNP) array enable elucidation of cryptic and submicroscopic genomic aberrations including copy number variations (CNVs) and regions of copy neutral loss of heterozygosity (CN-LOH) in Acute Myeloid Leukaemia (AML). Methods: We studied the AML genome using our custom CGH+SNP 180 K microarray which included additional custom probes for 49 genes with eleven genes covering every exon (TP53, DNMT3A, TET2, ASXL1, MLL, IKZF1, PAX5, EZH2, FLT3, NOTCH1 and ATM). Paired tumour and wild type (remission after induction sample obtained from the same patient) DNA were used to delineate germline variants. This study was carried out using Agilent SurePrint G3 CGH+SNP microarray platform in 41 AML samples. Tumour and matched wild type DNA aberrations and LOH intervals reports generated by Cytogenomics software version 2.9.2.4 were compared for all the cases. Matching aberrations present in tumour and matched wild type DNA were considered germline and changes present only in tumour were classified as tumour related changes. Results: After comparing tumour versus germline DNA, we found a total of 475 imbalances (n < 1 MB = 298, n 1-5 MB = 121, n 5-10 MB = 21, n 10-20 MB = 9 and n >20 MB = 26). We identified a 114 regions with losses, 330 regions with gains and CN-LOH were seen in 31 regions. The chromosomes most commonly gained were chromosomes and 2 (10.6%), 17 (10.3%) and 9 (8.5%) whereas chromosome losses were frequently seen in chromosome 7 (14.9%), X (13.2%) and 14 (12.3%). CN-LOH were mostly seen in chromosome 4 (35.5%) and 2 (12.9%). Array CGH+SNP detected abnormalities in the cases with poor metaphase quality and enabled identification of CN-LOH in four cases with normal karyotype. However, balanced translocations in five cases were not detected by array CGH+SNP. Conclusions: In summary, a combination platform of CGH+SNP provides invaluable insights in elucidation of large spectrum of genomic aberrations in AML which may demonstrate prognostic implications. Evaluation of tumour sample along with matched normal genomic enabled the delineation of somatic genetic lesion from the germline CNVs and CN-LOH.

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IMPACT OF NOTCH1 MUTATIONS IN B-CHRONIC LYMPHOCYTIC LEUKEMIA
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Introduction: The clinical heterogeneity that was described in chronic lymphocytic leukemia (CLL), with survival times ranging from less than 1–2 years to over 15 years, reflects its biological and molecular diversity. This could be variable in different ethnic patients groups around the world. Methods: We analyzed a cohort of 80 untreated chronic lymphocytic leukemia patients for novel genetic markers NOTCH1 in the context of molecular, immunophenotypic and cytogenetic data. The NOTCH1 mutation was identified by amplification refractory mutation system (ARMS) PCR and was confirmed by Sanger sequencing. Results: NOTCH1 c. 7544_7545 del CT mutation was detected in 16 out of 80 (20 %) cases of B-CLL. The patients with mutated NOTCH1 were refractory to therapy and express high risk of transformation into diffuse large B cell lymphoma. Overall survival was shorter in NOTCH1-mutated patients as compared to those unmutated. Conclusions: The presence of NOTCH1 mutation in CLL patients define a subset of patients with worse prognosis. The molecular testing for this mutation at B-CLL diagnosis is valuable for better stratification of those patients.
EVALUATING OF POSTTRANSPLANT RELAPSE RISK IN PATIENTS WITH ACUTE MYELOID LEUKEMIA USING THE GENE EXPRESSION PANEL.
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Introduction: It is known that up to 50 percent of cases of acute myeloid leukemia (AML) does not have informative genetic markers. At the same time, the studies of donor chimerism do not fully indicate the degree of tumor cells elimination and not always allow to estimate the risk of post-transplant relapse. Thus, finding of universal markers allowing for adequate therapy in post-transplant period, is quite important. WT1, BAALC, EVII and PRAME gene expression analysis is one of the possible approaches is this field.

Methods: Our study included 63 patients with AML who underwent allogeneic transplantation of hematopoietic stem cells (al-HSCT). In 24 patients myeloablative conditioning regimen were used, 39 – received reduced toxicity protocols. Assessing the levels of WT1, BAALC, EVII, PRAME gene expression and the level of chimeric transcripts was performed by means of RQ-PCR with normalization for ABL gene expression. Results: As based on gene expression in healthy donors, we have established cut-off overexpression (gene exp. / ABL exp. X100) values for the genes: WT1 – 250, EVII-10, BAALC– 20, PRAME - 200. For the present patient setting, we found no statistically significant differences in WT1, BAALC, EVII, PRAME genes expression between the patients with different FAB variants of AML. However we were able to identify a trend to higher values of PRAME and BAALC gene expression in patients with M1 variant, and WT1 gene values among patients with M4 variant of AML. In patients who underwent transplantation in relapse state, we have noted a significant overexpression of EVII (p = 0.006), WT1 (p < 0.001), BAALC (p < 0.001). A similar trend was observed for PRAME gene (p = 0.08). EVII gene overexpression was revealed for 6 patients (33%), WT1 in 13 cases (72%), BAALC in 10 patients (55%) and PRAME in 4 patients (22%). When comparing the data on chimeric transcripts expression, we detected a correlation between expression levels of the studied genes and chimeric transcripts (PML-RARα and RUNX1-RUNX1T1, p < 0.05) as well as with donor chimerism levels. At the same time, we did not find this relationship, when comparing with data about of expression of a CBFB-MYH11 chimeric gene. When evaluating the test sensitivity and specificity analysis we revealed a bellow-cutoff value of the expressed genes in presence of the chimeric transcript less than 2%. Conclusions: Evaluation of universal gene marker expression is an attractive approach for assessing efficiency of therapy in patients with AML. Thus, their use in early diagnostics of relapse in post-transplant period is quite promising. However, due to low specificity caused by basal expression in normal cells, further application of these markers is limited, when detecting minimal residual disease levels.

FETAL MICROCHIMERISM IN POST TRANSPLANT COMPLICATIONS AFTER HAPLOIDENTICAL BMT
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Introduction: Haploidentical transplantation of hematopoietic stem cells (haplo-HSCT) is a feasible therapeutic approach in patients lacking an HLA-identical donor. However, high risk of graft-versus-host disease (GVHD) is among major complications in haplo-BMT. Currently, there exist some data concerning associations between maternal microchimerism levels (detection of maternal cells in recipient’s blood) and increased risk for GVHD development. Meanwhile, the effects of microchimerism upon GVHD probability are still controversial. The aim of our study was to adapt a technique for quantitative evaluation of fetal (recipient-cell) and maternal (donor-cell) microchimerism and investigation of its effects upon actual risk of common complications after HSCT. Methods: Determination of microchimerism was carried out by real-time allele-specific polymerase chain reaction (AS RQ-PCR). The analytic panel included 20 SNP markers located at different chromosomes. Evaluation of PCR sensitivity was carried out using DNA mixtures from several established cell lines (HL60, K562, 293T, MOLT3, A549) at serial dilutions. The samples from 20 donor-recipient pairs were analyzed. The patients’ age ranged from 2 to 27 years. Results: The sensitivity thresholds for the SNP-based test panel ranged from 10^-4 to 10^-5. We have found out that detection of fetal microchimerism in the donor organism is associated with lower risk, or lower degree of acute GVHD (p=0.01). Moreover, we observed a trend to higher timeframe for transplant engraftment, and lower probability of full donor chimerism achievement, if the donor exhibited fetal microchimerism (p=0.12). The patients in our setting transplanted from donors with detectable fetal microchimerism had a tendency to a higher overall survival (p=0.14). However, we did not reveal any significant association between microchimerism levels (fetal and maternal), and higher probability of chronic GVHD development, as well as any differences in other posttransplant complications for recipients with maternal microchimerism. Conclusions: Evaluation of fetal microchimerism may be considered a useful and informative approach to selection of potential donors, or a method of GVHD prediction in haploidentical HSCT.
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CXCL10 GENE PROMOTER POLYMORPHISM -1447 A>G IS ASSOCIATED WITH SUSCEPTIBILITY TO MALARIA IN GHANAIAN CHILDREN

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Introduction: Plasmodium falciparum malaria kills nearly a million people annually. Over 90% of these deaths occur in children under five years of age in sub-Saharan Africa. In Ghana, malaria accounts for about 60% of all outpatient visits in public health facilities, with 40% of the affected being children under age 5 years. The disease accounts for 13.2% of all mortalities in Ghana and ranks fifth as the commonest cause of death in children under 5 years of age. The risk factors for severity of malaria pathogenesis and the wide variation in clinical manifestations of malaria are poorly understood. The influence of host genetics on susceptibility to P. falciparum malaria has been extensively studied over the past twenty years. We have recently shown that interferon gamma inducible chemokine, CXCL10, is a predictor of both human and experimental cerebral malaria severity. Additionally, we have reported that the -1447A>G polymorphisms in the CXCL10 gene promoter is associated with increased serum CXCL10 production, which is predictive of severity of malaria in Indian malaria patients. In the present study, we hypothesized that the -1447A>G polymorphism is present in Ghanaians and that it correlates with susceptibility to malaria. Methods: We assessed basic demographics that may impact our assessments including age, gender, hemoglobin levels, sickle cell status and CXCL10 polymorphism. We determined whether polymorphisms in the CXCL10 gene are associated with the clinical status of malaria patients. We assessed a single nucleotide polymorphism in the CXCL10 gene promoter (-1447A>G [rs4508917]) among 311 malaria and 62 non malaria cases using PCR-restriction fragment length polymorphism assay. Adjusted Odds Ratio (AOR) was used to find out if there was any association between CXCL10 promoter polymorphism -1447 A>G and susceptibility to malaria. Results: There was no significant difference with regards to hemoglobin level between malaria patients (12.6g/dL) and non-malaria patients (12.2g/dL), p=0.0484. The -1447A>G genotype of the CXCL10 gene was significantly associated with susceptibility to malaria (adjusted odds ratio =8.5, 95% CI=3.6-20.4, p< 0.0001). These results suggest that the -1447A>G polymorphism in CXCL10 gene promoter is associated with susceptibility to malaria in Ghanaian malaria children. These results further confirm what was found in the Indian cohort study that CXCL10 gene promoter -1447 A>G polymorphism was a marker for susceptibility to malaria. Conclusions: These results suggest that the -1447A>G polymorphism in CXCL10 gene promoter could be partly responsible for malaria outcomes in Ghanaian malaria children.

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DETECTION OF COPY NUMBER ALTERATIONS IN B-ALL USING MULTIPLEX LIGATION DEPENDENT PROBE AMPLIFICATION (MLPA)

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Introduction: The genes related to B-cell differentiation and other genes are frequently altered in B-ALL. Multiplex ligation dependent probe amplification (MLPA) can determine the copy number of many target sequences in a single multiplex PCR-based reaction. This study was carried out to find the prevalence of copy number alterations in B-ALL using MLPA technique. Methods: The untreated B-ALL cases were prospectively analyzed for copy number alterations in various genes like IKZF1, CDKN2A/B, PAX5, RB1, ETV6, JAK2, BTG1, CRLF2 and EBF1 over eleven months using MLPA (SALSA MLPA P335-B1 kit) in a tertiary care centre in Northern India. The MLPA was done using the genomic DNA as per the manufacturer’s protocol. Electrophoresis and quantification of amplicons was done on Genome Lab GeXP genetic analysis system and the data analysed using Coffalyser software. Results: The median age of 104 cases was 7 years (1-67). These included 62 males and 42 females. The gene deletions were seen in CDKN2A/B in 37 (35.6%) cases, PAX5- 33 (31.7%), IKZF1- 30 (28.8%), ETV6- 14 (13.5%), RB1- 13 (12.5%), JAK2- 11 (10.6%), BTG1- 6 (5.8%), and CRLF2 gene in 4 (3.8%) cases. The EBF1 gene showed duplication in 3 (2.9%) cases. Overall, the copy number alterations in the above mentioned genes were detected in 67 (64.4%) of the 104 cases. There was defect in only one of the genes in 32 (30.8%) cases, and 35 (33.7%) cases had more than one affected genes. Conclusions: Overall, MLPA could detect copy number alterations in at least one of the tested genes in 67 (64.4%) out of 104 cases of B-ALL. More than half of these cases were found to have more than one gene alterations.
DETECTION OF AML/ETO FUSIONS TRANSCRIPT IN ACUTE MYELOID LEUKEMIA IN

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Introduction: Acute myeloid leukemia (AML) is a heterogeneous bone marrow malignancy, and patients with the cytogenetic t(8;21) abnormality represent a subset with specific clinical and biological characteristics (WHO, 2008). The translocation fuses the AML1 gene (also called RUNX1) on chromosome 21 with the ETO gene (also referred to as the RUNX1T1 gene that encodes the CBFA2T1 protein) on chromosome 8. The criteria for the diagnosis differs from other AML patients; the leukemia cells show biological characteristics that are uncommon in other AML subsets, and the prognosis after intensive chemotherapy is better for these patients than for the majority of AML patients (WHO,2008). t(8;21) was the first cytogenetic abnormality discovered in AML (Rowley JD et al,1973), and today it offers a unique example of how a cytogenetic abnormality is used to define a distinct subgroup of patients. The AML1 gene has been reported to be involved in 39 different rearrangements and most of them have been detected in myeloid malignancies. In our institution, there was no study done as yet to detect the presence of t(8;21) in AML patients. Thus the objective of our study was to detect the AML1/ETO fusion transcript in some Sudanese patients with AML and to determine its relation with the FAB subtypes.

Methods: Study design and study area: This study is prospective hospital base cross sectional study, was conducted at Radiation and Isotopes Centre Khartoum (RICK), which is the main oncology Centre in Sudan, located in Khartoum state, serving almost all cancer patients in Sudan. Sample size Ninety four newly diagnosed AML patients presented during the period of the study (Feb/2008-Dec/2010) were enrolled. Samples were classified according to the French-American-British Cooperative Group (FAB), and the World Health Organization (WHO) systems. Samples Collection: Four hundred µl venous blood and one hundred µl bone marrow aspirates were obtained from each patient in evacuated (EDTA) containers. Sample collections were performed by trained personnel under medical supervision. Necessary precautions were taken to avoid sample contamination.

PCR Amplification: Taq polymerase cannot utilize RNA as template directly. Therefore, RNA (mRNA) was produced by reverse transcriptase using the standard protocol described by Dongen, et al(Dongen, et al. 1999). The product of this reaction, complimentary DNA (cDNA) was used as the template in RT-PCR reactions. In order to amplify expressed sequences, a DNA copy of the messenger RNA (mRNA) was produced by reverse transcriptase using the standard protocol described by Dongen, et al(Dongen, et al. 1999). The product of this reaction, complimentary DNA (cDNA) was used as the template in RT-PCR reactions. The following temperature profile used for cDNA reaction synthesis was 25°C for 10 min, 42°C for 45 min, 99°C for 3 min and held at 4°C. RT-PCR for detection of AML1/ETO was done according to the primers, protocol and criteria of the European BIOMED 1 Concerted Action for Standardization of MRD Studies in acute leukemia. The primer (alpha) combinations for 1° and 2° PCR for AML1/ETO fusion gene sequence and size to be amplified is shown in tables (1 and 2). Two round of PCR reaction were done; outer and nested PCR. The second round of PCR was used to increase the sensitivity of detection. PCR was carried out in 25 µl reaction mix containing 2-3 µl of cDNA in 0.5ml sterile micro centrifuge tube, forward primers A—B (AML1-A + ETO-B)100 µM final concentration, 10x PCR buffer, 100mM dNTPs, 25mM MgCl2 and 0.25 µl Tag DNA polymerase enzymes. 22-23µl of the pre-mixed was aliquot into each labeled tubes for outer reaction and 2-3µl of premixed reaction has been added. The final tube was the negative control. Positive control (KASUMI-I) was also included (gifted from Dr. Anne Sprout, clinical hematologist, Molecular Biology Department, Western General Hospital, Edinburgh, Scotland) table. To increase the sensitivity 1µl of outer product was added to the second round (nested PCR) using same volume, reagents and cycle conditions as for first round PCR, using the internal (nested) C ↔ D (AML1-C + ETO-D) reverse primers. Then the tubes have been placed into the PCR machine (Flexegene) and the following temperature profile was used for both outer and nested reactions: initial melting: 95°C for 30s, 94°C for 30s (melting), 65°C for 60s (annealing), 72°C for 60s (extension), number of cycles 35. Amplified of PCR products by Agarose electrophoresis: The PCR product was analyzed by weighing out 1.5g of Agarose powder into a 250ml conical flask, 100mL of 1x Tris-borate EDTA (TBE) was added, swirl to mix as described by Sambrook et al (Sambrook J, Russell D.W., 2001). The Agarose was microwave for about two minutes to be dissolved. Then it was left to cool down on the bench for 5 minutes at 60°C. 4µL of ethidium bromide (EtBr) was added and swirled to mix. The gel was poured slowly into the tank, and any bubbles were pushed away to the side using a disposable tip. After that, the comb was inserted and checked to be sure that it was correctly positioned. Then it was left for at least 30 minutes, with the lid on. 1x TBE buffer was poured into the gel tank to submerge the gel to 2—5mm depth, this was the running buffer. 3µl of loading dye was transferred using 10µl white tip into Para film paper and 7µl of PCR product was mixed together. Then the first well was loaded with standard DNA molecular weight size marker and the rest of the samples were loaded in order. It was run at 85 V for 45m, and then visualized on UV transilluminator and photographed gel using a digital camera. The bands were scored as not visible, weakly intensity (+), medium intensity (+++) or strong intensity (+++), compared with the molecular weight marker bands used. Results: Ninety-four patients diagnosed as AML by clinical presentation, conventional cytomorphology, and molecular studies in Radiation and Isotopes Centre, Khartoum, Sudan were analyzed. There were 50(53.2%)males and44(46.8%) females. The frequency of AML1/ETO was highest among those aged 10 to 19 years old. Most of patients harbor this fusion genes (68.4%) belonged to tribe of Middle Sudan. According to FAB classification the major subtypes were M2 comprised 28.7%, M3 were 21.3%, and M4 were 18.1%.The majority of AML patients were categorized as M2 subtypes (Figure 1). AML1/ETO transcript was detected in 38 (40.4%) out of94 patients studied where 2 were detected in children aged 2and 3 years. Based on morphology the transcript was detectedin those with AML-M2 (30/94) and AML-1(4/94). Agarose electrophoresis image for the AML1/ETO transcript was shown in (figure 2). Figure 1Shows AML samples according to FAB Figure 2:Represent Agarose gel with result from RT-PCR (nested PCR) for AML1/ETO fusion gene. Lane (1) 100 base-pair ladder size marker, lane (2) negative control, lane (3) positive control (Kasumi-1 cell line), lane (4) patient (1) negative result, and lane (5) patients sample (2 and 3) positive result.

Table 1: Show Primers (Alpha) and sequence for AML1/ETO RT-PCR for AML amplification

<table>
<thead>
<tr>
<th>Locus</th>
<th>Primer Code</th>
<th>Sequence 5' - 3'</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>AML1 F</td>
<td>AML1-A</td>
<td>CTACCAGGCAGCATGAAGAACC</td>
<td></td>
</tr>
<tr>
<td>AML1 F</td>
<td>AML1-C</td>
<td>ATGACCTCAGGTTGTCGGTCG</td>
<td></td>
</tr>
<tr>
<td>ETO R</td>
<td>ETO-B</td>
<td>AGAGGAGGCCCATTGCTGAA</td>
<td></td>
</tr>
<tr>
<td>ETO R</td>
<td>ETO-D</td>
<td>TGAAGCTGTTCTTGAGCTCT</td>
<td></td>
</tr>
</tbody>
</table>

Table 2: Show sizes (bp) of PCR products obtained using ALPHA primers for AML1/ETO

<table>
<thead>
<tr>
<th>Primer combination</th>
<th>A+B(First round)</th>
<th>C + D</th>
<th>A+(B)+(C+D)(Nested)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AML1/ETO</td>
<td>395</td>
<td>260</td>
<td>260</td>
</tr>
</tbody>
</table>
Introduction: The primary purpose of a multiplex reverse transcriptase polymerase chain reaction (RT-PCR) platform is to comprehensively screen for recurrent genetic abnormalities. The aim of this study was to draw an additional role of this platform in the initial evaluation of patients with acute leukemia. Methods: A total of 945 consecutive specimens from patients with acute leukemia were tested for molecular genetic alterations using a commercial multiplex RT-PCR kit (HemaVision, DNA Technology) from July 2010 to January 2015. Our laboratory approach was to subsequently assess the FLT3, NPM1, and CEBPA mutations, along with MLL-partial tandem duplication for patients with acute myeloid leukemia (AML) who displayed no abnormal bands in multiplex RT-PCR. Results: Cyto genetics failed in 7 samples (0.7%) and yielded an insufficient number of metaphases (< 20) in 74 samples (7.8%), whereas multiplex RT-PCR was successfully performed in all samples. Cryptic translocations were detected in 20 samples (2.1%) including TEL-AML1 (n=10), BCR-ABL1 (n=5), PML-RARA (n=2), MLL-MLLT10 (n=2), and MLL-MLLT4 (n=1). Multiplex RT-PCR also provided molecular information on 7 submicroscopic aberrations (0.7%), including SIL-TAL1 (n=3) and SET-NUP214 (n=4). This technique further provided information on potentially significant, but rare, aberrations or combinations in 14 cases (1.5%), including BCR-ABL1-positive AML (n=3), CBFB-MYH11 variant D (n=2), CBFB-MYH11 variant C (n=1), BCR-ABL1 (n=1), coexistence of CBFB-MYH11 and BCR-ABL1 (n=1), ETV6-MN1 (n=1), and RUNXI-RUNXIIT1 and ETV6-MN1 (n=1), RUNXI-MDS1 (n=1), FUS-ERG (n=1), ETV6-PDGFRB (n=1) and NPM1-MLF1 (n=1). However, there were 3 cases (0.3%) in which multiplex RT-PCR failed to detect fusion genes corresponding to recurrent translocations, including inv(16)(p13.1q22) (n=1), t(11;19)(q23:p13.3) (n=1), and t(9;22)(q34;q11.2) (n=1). A subset of patients among 422 multiplex RT-PCR-negative AML were able to be possibly reclassified into a favorable risk group, either owing to FLT3-ITD™/NPM1™ (13.7%, 58/422) or CEBPA double mutation (6.7%, 21/312).

Conclusions: The present study indicates that the use of multiplex RT-PCR in patients with acute leukemia generated relatively robust results compared to conventional cyto genetics. Multiplex RT-PCR could provide additional information, including cryptic translocations and rare aberrations, as well as suggests guidance on the selection of AML candidates for mutation screening. Multiplex RT-PCR and subsequent mutation screening can be useful for therapeutic preparation at the early diagnostic stage.

CALRE TICULIN MRNA EXPRESSION AND CLINICO-PATHOLOGICAL CHARACTERISTICS IN ACUTE MYELOID LEUKEMIA

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Introduction: Calreticulin, encoded by CALR, is a multifunctional protein with roles in calcium homeostasis and chaperoning molecular processes. Although some studies have examined the association between cs-CALR and AML, the characteristics and clinical relevance of CALR mRNA expression in AML are not well known. This study aimed to evaluate calreticulin mRNA expression levels in acute myeloid leukemia (AML) compared with other hematologic malignancies, and to investigate the clinicopathological characteristics associated with expression in AML patients.

Methods: The study group included 43 patients diagnosed with AML, 57 with other hematologic malignancies, and 21 benign hematologic conditions. The relative quantification of CALR mRNA was measured using real-time PCR targeting CALR gene in exon 7-8 boundary (Taqman Gene Expression Assays, Life Technologies). Results: The CALRmRNA expression was significantly higher in AML compared with other hematologic malignancies (P<0.0001). There was no difference in CALR mRNA expression between AML subgroups by karyotype (P=0.3201). There were found in age, white blood cell counts, platelet counts, bone marrow blast percentage, calcium, or lactate dehydrogenase between the high and low CALR groups (CALR mRNA ≥1.2 fold and < 1.2 fold, respectively), although hemoglobin and sex differences were observed. Overall survival and disease-free survival were similar between the two groups (P>0.05). Conclusions: AML patients showed higher CALR mRNA expression levels than those with other hematologic malignancies, although no association of CALR expression with clinicopathological characteristics was observed, except for hemoglobin and sex. The clinical relevance of CALR expression in AML remains to be clarified in a larger cohort.

Poster Presentation Abstracts
CLINICAL SIGNIFICANCE OF NUP98 rearrangement in acute myeloid leukemia

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Introduction: The nucleoporin 98-kDa gene (NUP98), maps to chromosome 11p15.5, encodes a nucleoporin 98-kDa protein (Nup98), which is a part of the nuclear pore complex. NUP98 rearrangement has been reported in various hematological malignancies such as acute myeloid leukemia (AML), myelodysplastic syndrome, T-cell acute lymphoblastic leukemia, and chronic myeloid leukemia. At least 28 different partner genes are known to be fused with NUP98. For establish the distribution and clinical impacts of NUP98 rearrangement in AML, we performed fluorescence in situ hybridization (FISH) test to screen the rearrangement and reviewed clinicopathologic features of the cases. Methods: Out of 455 newly diagnosed AML patients between October 2008 and March 2014, six patients with 11p13-15 abnormalities selected for FISH to screen NUP98 rearrangement. Bone marrow (BM) aspiration sample was cultured in mitogen-free media for 24 hours. Metaphase chromosomes were analyzed using Giemsa-trypsin-Giemsa-banding technique. FISH was performed using NUP98 break probes (Kreatech Diagnostics, Amsterdam, the Netherlands), by following manufacturer’s instructions. Results: There were two patients (33%) with NUP98 breakage detected by FISH. One patient with 46,XX,t(10;11)(q22;p15)[20] was female and diagnosed AML at 81 years. She showed leukopenia and anemia at diagnosis. BM study showed 32.3% of leukemic blast and dysplasia of neutrophils and erythroid precursors. Immunophenotyping study revealed CD13, CD33, CD34, CD38, CD117, HLA-DR, and MPO positivity. FISH detected a break-apart signal in 82.6% interphase cells, indicating a rearrangement involving NUP98. She diagnosed with AML with maturation and died 12 months after diagnosis due to sepsis and decreased blood pressure. The other patient with 46,XX,t(7;11)(p15;p15)[20]/NUP98-HOXA9 was female and diagnosed at 70 years. Initially she showed leukopenia and thrombocytopenia. Leukemic blasts (15.2%) and dyspoietic megakaryocytes (15%) were observed in BM. The blast was positive for CD13, CD33, CD34, CD38, and MPO. A break-apart signal was observed in 71.2% of interphase cells on FISH study. She diagnosed AML with maturation and received salvage chemotherapy, but died 10 months after diagnosis due to decreased blood pressure and acute renal failure. Conclusions: NUP98 rearrangement is known for association with poor prognosis. Our patients also cannot achieve complete remission and showed poor prognosis, emphasizing the prognostic impact of NUP98 aberrations. FISH is useful technique to detect NUP98 rearrangement in AML.

AUTOMATIC IDENTIFICATION OF NORMAL, REACTIVE LYMPHOCYTES AND LEUKEMIC LINEAGE BLAST CELLS FROM DIGITAL PERIPHERAL BLOOD CELL IMAGES

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2CoDAlab. Universitat Politècnica de Catalunya. Barcelona Spain

Introduction: Peripheral blood (PB) examination in leukemic patients provides important information for their initial diagnosis and management. Most automatic morphologic analysis equipments underestimate blast cells in PB, mistaking them as reactive (RL) or normal (N) lymphocytes. Likewise, they do not distinguish leukemic lineage of blast cells. The objective was to apply a methodology for the automatic classification of myeloid (MB) or lymphoid blast (LB) cells in acute leukemia, separating them from RL and N lymphocytes. Methods: The work was carried out in two stages: 1) System training: A training set (TS) of 1527 digital cell images from PB smears stained with MGG and obtained in the Cellavision DM96 were analyzed. From them, 500 were MB and 479 LB from 13 acute myeloid leukemia (AML) and 6 acute lymphoid leukemia (ALL) patients respectively, 347 RL from 30 infectious mononucleosis patients and 201 N lymphocytes from 25 healthy patients. We segmented regions of interest (ROI) of the cells and from these ROI, 13 geometric and 6422 color-texture features were extracted. We selected the 65 most significant features by information theory to maximize the relevance and minimize the redundancy. Support vector machines method was used for the classification. 2) Validation of the system: A new an independent set of images (373) from 42 patients (5 AML, 5 ALL, 12 with RL and 20 healthy) was used for the validation. Results: We segmented 3 different ROI in the cells: nucleus, cytoplasm and peripheral zone. In the first stage, the global classification accuracy considering the training set was 90.1%, with true-positive classification rates of 85% for MB cells, 87% for LB cells, 97% for RL and 98% for N lymphocytes. In the validation stage, the global accuracy was 81.5% and the true-positive classification rates were 76% for MB, 71% for LB, 90% for RL and 87% for N lymphocytes. Conclusions: The method allowed the automatic discrimination of leukemic lineage blast cells obtaining high accuracy in the classification of these blast cells, RL and N lymphocytes. The validation results obtained in individual patients were encouraging to extend the method as a support tool for acute leukemia diagnosis.
A NEW METHOD TO ASSESS COMPETENCE IN MORPHOLOGIC PERIPHERAL BLOOD DIAGNOSIS
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1Hospital Clinic Barcelona, Spain, 2Spanish Society of Hematology and Hemotherapy (SEHH) Standardisation Committee Madrid, Spain

Introduction: A new scoring method to assess competence in peripheral blood (PB) morphology evaluation was established taking into account the results obtained in the External Quality Assessment Scheme for PB morphology (EQAS-M) during the last 12 years. Objectives: To validate the application of the scoring method developed with their use to assess competence in morphologic diagnosis using PB smears in a six months e-learning course addressed to clinical laboratory professionals. Methods: The main purpose of the method was to assess, in an objective way, the skills of the participants to recognize the target cells present in the different PB smears (one monthly, six in total). We established three levels of competence (high, low or null) with respect to the results of the score. A total of 33 clinical pathologists participated in the first “Competence evaluation in PB morphologic diagnosis” e-learning course in a Moodle platform. We used some theoretical contents about haematological diseases, PB digital images and six PB smears stained with MGG obtained from patients with the following diagnosis: Bernard Soulier syndrome (BSS), chronic myeloid leukemia (CML), malaria infection, hairy cell leukemia (HCL), acute myeloid leukemia (AML) and promyelocytic leukemia (PL). Results: A total of 96% of the professionals identified the presence of giant platelets in BSS film, showing high competence (HC) for this morphologic diagnosis. In addition, a number of 92%, 92% and 100% of the participants showed HC to detect malaria, AML and PL respectively. Of the method was to assess, in an objective way, the skills of the participants to recognize the target cells present in the different PB smears (one monthly, six in total). We established three levels of competence (high, low or null) with respect to the results of the score. A total of 33 clinical pathologists participated in the first “Competence evaluation in PB morphologic diagnosis” e-learning course in a Moodle platform. We used some theoretical contents about haematological diseases, PB digital images and six PB smears stained with MGG obtained from patients with the following diagnosis: Bernard Soulier syndrome (BSS), chronic myeloid leukemia (CML), malaria infection, hairy cell leukemia (HCL), acute myeloid leukemia (AML) and promyelocytic leukemia (PL). Results: A total of 96% of the professionals identified the presence of giant platelets in BSS film, showing high competence (HC) for this morphologic diagnosis. In addition, a number of 92%, 92% and 100% of the participants showed HC to detect malaria, AML and PL respectively. With respect to the CML smears, 78% of the participants obtained HC, and 18% low. In addition, the higher percentage of professionals showed a null competence level was found in HCL smears (11%) Conclusions: The application of the method described showed that: 1) hairy cell leukemia was the most difficult pathology to identify by the participants using only morphology features and 2) could be useful to assess competence in morphologic peripheral blood diagnosis.

502 STRAIGHTFORWARD IDENTIFICATION OF MASKED POLYCYTHEMIA VERA BASED ON PROPOSED REVISION OF WHO DIAGNOSTIC CRITERIA FOR BCR-ABL1-NEGATIVE MYELOPROLIFERATIVE NEOPLASMS
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Introduction: Because of prominent thrombocytosis, some cases of early-stage polycythaemia vera (PV) may mimic essential thrombocythaemia (ET). A recently proposed revision of the 2008 World Health Organization (WHO) diagnostic criteria for BCR-ABL1-negative myeloproliferative neoplasms (MPN) has highlighted the diagnostic pitfalls in these cases and re-introduced the clinical entity of “masked” PV. Methods: We retrospectively reviewed the hematologic data of 84 patients with ET who were available for assessment of JAK2, MPL and CALR mutational status, from January 2003 to April 2013. Results: We found 11 (13.1%) patients with hemoglobin (Hb) or hematocrit values ranging between the WHO 2008 and proposed 2014 criteria. All 11 patients had the JAK2 V617F mutation, but neither the MPL nor the CALR mutations, suggesting that they might have features of PV rather than ET. We then reviewed their bone marrow histomorphology and identified 2 (2.4%) patients who fulfilled the proposed revision of criteria for PV. Bone marrow morphology of these patients showed the trilineage proliferation and an increased number of pleomorphic megakaryocytes. Patient 1 was a 36-year-old man with a Hb level of 17.6 g/dL, leukocyte count of 12.3x10^9/L, and platelet count of 577 x10^9/L, and normal serum erythropoietin level. He had been diagnosed with ET and received anagrelide therapy. Two months later, he was additionally diagnosed with non-ST-segment elevation myocardial infarction and underwent off-pump coronary artery bypass. Patient 2 was a 63-year-old man with a Hb level of 17.9 g/dL, leukocyte count of 19.3x10^9/L, and platelet count of 1,557 x10^9/L. He had been diagnosed with ET and received hydroxyurea as well as antithrombotic therapy. At the time of this writing, these patients have been doing well for 2 years and 1.5 years, respectively. Conclusions: The simplified and clarified proposed 2014 WHO criteria can facilitate the diagnosis of masked PV. Using the revised criteria, we were able to identify 2 patients with masked PV, who had been diagnosed with ET according to the 2008 WHO criteria. In conclusion, the 2014 proposed revision of WHO criteria for BCR-ABL1-negative MPN can ensure a straightforward identification of masked PV.
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STUDY OF MALARIA CASES IN THE PEDIATRIC DEPARTMENT OF KORLE BU TEACHING HOSPITAL, GHANA

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1University Of Ghana Medical School Accra, Ghana, 2Child Health Department, KBTH Accra, Ghana, 3Child Health Department, KBTH Accra, Ghana, 4Chemical Pathology Department, UGMS Accra, Ghana, 5Dept of Parasitology, Nnamdi Azikiwe University Awka, Nigeria, 6University of Ghana Medical school Accra, Ghana

Introduction: Malaria kills about one million children, under five years of age, each year worldwide, with nine out of 10 deaths occurring in sub-Saharan Africa. This study was carried out to determine the incidence of malaria in the pediatric department of Korle Bu Teaching Hospital from January 2014 to October 2014, and to compare available diagnostic tests for malaria. Methods: 978 suspected cases of malaria (507 males and 471 females, aged 1 day – 12 years), attending the OPD and admitted in the ER were included in this study. 1.0 ml of blood sample was collected into EDTA bottle from all clinically suspected cases of malaria. Thick and thin smears were prepared, stained and examined using the standard procedure. Subsequently, the blood sample was subjected to antigen detection using the Malaria pLDH/HRP Test kit according to the manufacturer’s instructions. The species of the parasite were noted. Results: 51 cases out of 978 suspected cases were positive for malaria, with an incidence of 5.2%. Out of these 40 (78.4%) were positive for Plasmodium falciparum, 5 (9.8%) were positive for Plasmodium malariae, 2 (3.9%) were positive for Plasmodium ovale, and 4 (7.8%) were positive for both Plasmodium falciparum and Plasmodium malariae. The Malaria pLDH/HRP Test kit detected 51 positive cases compared with the blood smear study, which detected 41 cases. 36 cases were detected both by the Malaria pLDH/HRP Test kit and blood smear study. 15 cases were positive by the Malaria pLDH/HRP Test kit but not by the blood smear study. 5 cases detected to be positive by the blood smear study were found to be negative by the Malaria pLDH/HRP Test kit. 937 cases were negative both by the Malaria pLDH/HRP Test kit and the blood smear study. Among 51 positive cases, 35 were males with a percentage of 68.8% as compared to females (31.4%). The sensitivity, specificity, positive predictive value, negative predictive value, and diagnostic efficiency of the Malaria pLDH/HRP Test kit when compared to microscopy, were 87.5, 96.8, 80, 98.9, and 96%, respectively. Conclusions: The incidence of malaria in this present study was 5.2%. The sensitivity of Malaria pLDH/HRP Test kit is very close to microscopy and it does not require highly skilled personnel to perform or interpret results. Therefore, Malaria pLDH/HRP Test kit is a simple, sensitive, and effective diagnostic test for P. falciparum, P. malariae, P. vivax and P. ovale malaria.

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CONTRIBUTION OF THE PERIPHERAL BLOOD CELL IMAGES OBTAINED IN THE CELLAVISION DM96 TO THE DETECTION OF UNUSUAL INCLUSIONS INSIDE NEUTROPHILS

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Introduction: Microscopic examination of peripheral blood (PB) cells is an important diagnostic tool. CellaVision DM96 (DM96) obtains digital images of the blood cells. We evaluated the contribution of these digital PB cell images obtained in the DM96 to detect different unusual inclusions inside neutrophils. Methods: Blood samples were obtained from the routine workload of the Core laboratory of the Hospital Clinic of Barcelona. Samples were analyzed by a cell counter Advia 2120 and PB films were stained with May Grünwald-Giemsa within 4 hours of blood collection. The slides were loaded into the Cellavision DM96 obtaining digital images of the blood cells. Morphologic examination of the blood smears was performed using the digital images at high magnification. Results: Morphological analysis of PB using digital images showed that Döhle bodies were the most frequent inclusions detected inside neutrophils. Unexpected inclusions were seen in the following cases: 1) Pseudo Howell-Jolly inclusions were detected inside neutrophils in at least 100 patients (P) receiving a) antiviral therapy with drugs acting on the genoma replication, b) chemotherapy, immunosuppressive therapy or both.; 2) Intracytoplasmic cocci type microorganisms in 1 patient, in which blood cultures were positive for Streptococcus gallolyticus; 3) Abundant vacuole-like cytoplasmic inclusions with compression of the nucleus in neutrophils associated to cryoglobulinemia and IgG-kappa monoclonal gammopathy of undetermined significance (1 P); 4) Neutrophil vacuolation associated to sepsis (2 P); 5) Platelet phagocytosis by neutrophils EDTA dependent (1 P); 6) Erythrophagocytosis associated to cold agglutinin disease (1 P) or Epstein-Barr virus IgM-mediated hemolytic anemia (1 P); 7) Giant and abnormally staining granules associated to Chédiak-Higasi syndrome (1 P) and 8) Neutrophils containing randomly distributed, single or double blue-gray large cytoplasmic inclusions, some having a spindle or crescent shape in the May-Hegglin anomaly (1 P). Conclusions: Automated peripheral blood evaluation using digital cell images provided by the DM96 is a valuable support tool facilitating the detection of unusual inclusions inside white blood cells that is essential for the morphological initial diagnosis.
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COMPLETE BLOOD COUNT CHANGES IN PATIENTS WITH DENGUE VIRAL INFECTIONS AT HOSPITAL UNIVERSITÁRIO DA UNIVERSIDADE DE SÃO PAULO - SÃO PAULO-BRAZIL
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Introduction: Dengue viral infection is transmitted mainly by Aedes aegypti mosquito and there are four distinct serotypes virus (DENV1, DENV2, DENV3, and DENV4) which can result in dengue fever and dengue hemorrhagic fever.1 The risk of developing dengue infection is present in more than 125 tropical and subtropical countries.2 Over the last year (2014), in Brazil, the number of cases confirmed by serologic testing (ELISA-IgM) was 572,308.3 According to the World Health Organization 2009, one of the most important resources in the detection and management of dengue in laboratory investigation is the Complete Blood Count (CBC).4 In March 2014, in a Dengue epidemic, we observed in our lab an increase of number of abnormal blood counts which probably were associated with Dengue.

Methods: In order to better define the CBC’s profile in seropositive Dengue patients, the objective of this study was to identify what kinds of CBC’s changes are more common in seropositive patients using Sysmex XT-2000i Automated Hematology Analyzer.

Results: In this study, 152 individuals were screened for dengue infection and 116 (76.3%) of them were found to be seropositive. The results of seropositive Dengue patients were: 44/116 (37.9%) had thrombocytopenia, 56/116 (48.3%) leukopenia, 44/116 (37.9%) ‘Atypical Lympho/Blast’ flag and 27/116 (23.3%) had pseudo basophilia. The number of patients (48.3%) leukopenia, 44/116 (37.9%) ‘Atypical Lympho/Blast’ flag were associated with Dengue.

Conclusions: The typical laboratory findings were leukopenia, thrombocytopenia and as a viral infection, it causes a specific immune response, atypical lymphocytes, which generated an ‘Atypical Lympho/Blast’ flag and pseudo basophilia. The causes of pseudo basophilia should be further investigated in future studies.

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SCREENING FOR MALIGNANT LYMPHOID CELLS ON PERIPHERAL BLOOD FILMS - A COMPETENCY ASSESSMENT STUDY IN A LARGE TERTIARY TEACHING HOSPITAL
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Introduction: The frontline technologists’ ability to recognize occult malignant lymphoid cells on peripheral blood films (PBF) is of critical diagnostic importance. The objectives of the study are (i) to evaluate the competence of practicing technologists in the detection of lymphoid malignancy on PBF, and (ii) to compare the relative diagnostic performance between an automated digital imaging system (CellaVision®) and the manual blood film review (MBFR) method.

Methods: 21 technologists working in the core hematology laboratory participated in a PBF quiz, which included 11 anonymous patient PBF slides (7 with malignant cells: chronic lymphocytic leukemia (CLL-2), marginal-zone lymphoma (MZL-1), follicular lymphoma (FL-2), lymphoplasmacytic lymphoma (LPL-1), plasma cell leukemia (PCL-1), B acute lymphoblastic leukemia (ALL-1), and 4 with reactive lymphocytes). The participants were required to report their findings/opinions: (i) Presence/absence of malignant cells, (ii) Assessment of likely diagnosis: lymphoma, ALL, PCL or reactive/normal condition. The quiz was first conducted on CellaVision®, and after case randomization and one-month washout period, repeated on MBFR.

Results: CellaVision® - Sensitivity: mean 81.7%, CI (75-88.5%); Specificity: mean 100% CI (100%). MBFR - Sensitivity: mean 83.5%, CI (75.9-89.2%). Specificity: mean 100% CI (100%). (Between-method comparison, p=ns). The correct classification rate for lymphoid malignancies: CellaVision® - CLL1 (10/18), CLL2 (4/18), FL1&2 (18/18), MZL (17/18), PCL (18/18), ALL (18/18); MBFR – CLL1 (11/18), CLL2 (9/18), FL1 (18/18), FL2 (14/18), MZL (16/18). Paired comparison revealed no significant difference between the two methods except for FL2, which had few circulating lymphoma cells on PBF (< 10% lymphoma cells; lymphocyte count 1.6 giga/L). More technologists labelled correctly FL2 as lymphoma by CellaVision® than by the MBFR method (p=0.034). The majority of participants misidentified 1 or 2 CLL cases as normal/reactive conditions by both CellaVision® and MBFR (selected cases had mild lymphocytosis and few smudge cells). Sub-analysis excluding CLL cases revealed more technologists identified correctly the remaining lymphoid malignancies by CellaVision® than MBFR (17/18 vs. 12/18, p=0.035).

Conclusions: Overall, the participants achieved good diagnostic accuracy. CellaVision® is a safe supplementary/alternative technology to MBFR in the detection of lymphoma cells, and may have an advantage over MBFR for the recognition of low level circulating lymphoma cells on PBF.
A UNIQUE FORUM OF GENERAL HEMATOLOGY
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Introduction: The Forum of General Hematology was established in Israel 12 years ago and meets every 4 months. There are currently about 200 members representing 42 hematology laboratories from all over the country. The participants compare results of identical morphological slides provided in one meeting and they are discussed and validated at the next Forum meeting. Thus a form of quality control for morphology is created. The aims of the Forum: To provide hematology morphology training To create a Morphology Quality Control system. To comparison and synchronize results of the participating hematology laboratories. To discuss new hematological methods and common laboratory problems. Provide a forum for discussing cutting edge techniques, different hematological subjects and to encourage cooperation between laboratories. Methods: The Forum meets every 4 months. The laboratory representatives receive 4/5 identical pathological blood films for analysis. The results are sent to the organizer of the Forum. Statistical evaluations and anonymous results for all participants are returned to each participant. These results are discussed in the next meeting when participants receive new blood films to characterize. In addition, hematological topics and problems are discussed. Results: The comparison of slides carried out in the Forum, offers a validation test for the individual laboratories. The laboratories are required to uphold these standards which are those of the Israel Laboratory Accreditation Authority. These meetings create uniformity in the report of results in Israeli laboratories. Enables the sharing of knowledge as well as a quality control program for blood morphology. Conclusions: The Forum is a meeting of great importance for improving professionalism of the participating laboratories. The objectives of the Forum is studying the morphology, fertilization and increasing the skill of laboratory staff. By comparing morphological results and their definition a national quality control program is certificates are awarded annually to participants by the Forum with the Israeli Hematology Association. This Forum has proven that it upholds laboratory standards and professionalism; it improves networking and inter-laboratory cooperation. The satisfaction and every growing participating members are witness to the importance of the Forum.

DIAGNOSING CHÉDIAK-HIGASHI, ADDED VALUE OF THE LABORATORY.
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Introduction: Chédiak-Higashi syndrome (CHS) is an autosomal-recessive disorder with hypopigmentation caused by mutations in the Lysosomal Trafficking Regulator gene. The disease is characterized by recurrent infections, partial albinism, coagulation defects due to abnormal platelet aggregation and neurological manifestations (e.g. progressive peripheral neuropathy, mental retardation). If not treated with hematopoietic stem cell transplantation (HSCT), most patients die within the first 10 years of life from hemophagocytic lymphohistiocytosis (HLH). So far, 500 patients have been reported. Methods: Case report: A 2-months old girl, first child of consanguin parents was admitted in our hospital with a history of high fever, anorexia, neutropenia and hepatosplenomegaly. Laboratory results showed a profound thrombopenia, anemia and neutropenia. Increased liver enzymes and disturbed coagulation was compatible with liver function failure. Results: To exclude acute leukemia, a bone marrow puncture was performed. Bone marrow analysis revealed normal cellularity with left shift in the myeloid lineage and an increased percentage of myeloblasts (11%). Striking was the presence of abnormal inclusion bodies and vacuolization (mainly in the myeloid lineage, only limitedly observed in the lymphocytes). These inclusions were also found in the peripheral blood, but less conspicuous due to the neutropenia. CHS-like granules have also been described in myeloid cells in acute myeloid leukemia, but not in the lymphocytes. Therefore, acute leukemia was excluded. Further clinical investigations showed partial oculocutaneous albinism, which was confirmed by ophtalmology and light microscopic analysis of silvery hairs (with presence of evenly distributed large melanin granules and bright and polychromatic refingrence under polarized light). Flowcytometric analysis showed reduced NK cell cytotoxicity as illustrated by a reduced CD107a upregulation after stimulation with k562 cells and a lower percentage killing compared to the healthy control. The clinical presentation together with laboratory abnormalities (including low fibrinogen) were compatible with acute HLH. Genetic testing for CHS1/LYST mutations is pending. Conclusions: The finding of cytopenia and abnormal blood cell inclusions in a context of infection is sometimes the initial observation leading towards the diagnosis of CHS. Early diagnosis in CHS is important to allow therapeutic intervention by HSCT before HLH occurs.
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NAEGELIA FOWLERI INDUCING PRIMARY AMOECIC MENINGOENCEPHALITIS: A RARE CASE OF SURVIVAL
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Introduction: Primary amoebic meningoencephalitis (PAM) is a rare and almost always fatal disease that is secondary to Naegleria fowleri, a freshwater thermophilic amoeba. This case is remarkable as it involves an adolescent female who survived PAM. Methods: The electronic medical record and Wright-Giemsa stained CSF cytospin slides were reviewed. A PubMed search for reported cases of PAM survival was performed. Results: A previously healthy 12 year old female presented to the emergency department (ED) with a three day history of headache, lethargy and fever (up to 103F). In the 12 hours prior to presentation, she developed nausea and vomiting. The patient has a history of swimming at a fresh water park a week ago. A computerized tomography scan of her head showed normal findings. A CBC identified mild neutrophilic leukocytosis and normocytic anemia. Lumbar puncture was performed and identified predominantly neutrophils. The CSF Wright stain was positive for numerous organisms (see Figure 1). Senior medical technologists in hematology and microbiology identified the organisms as morphologically consistent with the diagnostic trophozoite stage of Naegleria species and informed the ED staff approximately one hour after receiving the CSF specimen. Actively motile trophozoites morphologically consistent with naegleria were also identified on a wet mount of the CSF. Infectious Disease was consulted immediately and the patient was treated with amphotericin, rifampin, azithromycin, fluconazole and dexamethasone, to control for cerebral edema. The CDC was contacted and Miltefosine, an investigational medication, was started on the second day of hospitalization. Subsequent evaluation of the cerebrospinal fluid included a CSF amoeba culture which identified amoebae, consistent with Naegleria species. Real time PCR, performed at the Centers for Disease Control, was positive for Naegleria fowleri and excluded Acanthamoeba and Balamuthia. Conclusions: Our patient made a full recovery after 52 days of hospitalization. Follow up magnetic resonance imaging reports of the brain, one year after successful treatment of PAM, identified subtle gliosis of both cerebral hemispheres involving the cortex and white matter. Successful treatment of PAM is a rare occurrence and may rely on rapid recognition, communication with the CDC and appropriate treatment including the use of Miltefosine. Therefore, this illness should be considered in the appropriate clinical setting especially in Southern states during the summer months.

Introduction: The Mean Platelet Volume (MPV) is a platelet parameter obtained with ease from most hematology cell counters. An increase in MPV has been associated with a variety of disease states - idiopathic thrombocytopenia, adverse coronary events, intestinal tuberculosis, sepsis and other inflammatory states. The MPV is low in Wiskott Aldrich syndrome and in the bone marrow failure syndromes. There is a paucity of literature on the variables affecting MPV in healthy children which makes it difficult to interpret this parameter in disease states in the pediatric population. This study was undertaken to determine the effect of age and sex on the MPV. Methods: Dipotassium EDTA blood was collected by venepuncture from 336 children clinically healthy children aged between 15 days -18 years after obtaining consent from the parents. The 190 males and 146 females were grouped as < 1 year, 1-< 5 years, 5<-10years and >10 years with 110,109, 48 and 69 cases in each category respectively. The complete blood cell counts and MPV were obtained on the LH 750 Beckman Coulter Hematology Cell Counter within 4 hours of collection. Subjects with history of recent hospitalisation or intake of medicine within the last 14 days and platelet counts < 100x10^3/L were excluded. The MPV was compared between the age groups, gender and CBC parameters. Results: The median MPV was 9.17(IQR-8.1-10) fl. The mean MPV in males was higher, 9.2 vs. 9.1 fl in females, however the difference was not significant (p=0.462).The lowest MPV were seen in children < 5 years. The mean MPV in the age groups mentioned above were 9, 8.8, 9.5 and 9.8 fl respectively and varied significantly with age (p<0.001). MPV correlated with platelet count (r=-.58), hemoglobin (r=-.29), hematocrit (r=.32) and MCV (r=.19) all with p<.001. Conclusions: The MPV in clinically healthy infants is lower than that of older children .MPV increases with age in childhood. The MPV does not vary significantly with sex. As is previously known in children with low platelet count, the inverse relationship between MPV and platelet counts is also seen in healthy children with normal platelet counts. Age specific ranges need to be determined in the pediatric population in order to use this parameter in distinguishing the disease state from the normal to improve its utility.
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REPRODUCIBILITY OF MANUAL PLATELET ESTIMATION
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Introduction: Accuracy of automated platelet estimation is low with large variability when the platelet count is below 100 x 10^9/L. Therefore, manual platelet estimation is a standard of care in these settings. The reproducibility of manual platelet counting has not been well studied. The aim of this study is to assess the reproducibility of manual platelet estimation.

Methods: Peripheral blood films of patients with platelet count of less than 100 x 10^9/L were retrieved and prospectively given to four observers to perform independent blinded manual platelet estimation using a pre specified method (The average of platelet counts in 10 fields using 100 x objective multiplied by 20). Data included hemoglobin level (Hb), mean red cell volume (MCV), automated platelet count (PLT), and observers manual platelet estimation (Obs [1-4]). Data were analyzed using interclass correlation (ICC) as a method of reproducibility assessment.

Results: The mean Hb and MCV were 13.7 (SD 3.3) and 60.8 (SD 11.2) respectively. The mean PLT was 58 (SD 30.3). The mean estimated manual platelet counts by the four observers [1-4] were 127 (SD 43.9), 107 (SD 45.4), 84 (SD 35.1) and 108 (SD 35.9) respectively. The ICC across the four observers was 0.84 indicating a very good agreement. The median difference of the two most experienced observers (Obs 3 and 4) was 0 (range: -64 to 78) which included clinically relevant differences (Figure 1). The level of platelet estimate by the lesser experienced observer predicted the disagreement (P=0.037). There was no significant difference in ICC among different observer pairs (P=0.42). Conclusions: There is a very good agreement between the observers; however, differences in some instances were large and clinically relevant. The results need to be confirmed in a larger prospective study.

Figure 1: Modified Bland-Altman Plot (Rater 3 vs. Rater 4)

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NEONATAL PSEUDOTHROMBOCYTOPENIA: SAMPLE CLOTTING AND PLATELET CLUMPING ASSOCIATED WITH SAMPLE VOLUME AND COLLECTION METHODS
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Introduction: Thrombocytopenia is a common laboratory finding. Although neonatal thrombocytopenia may be severe and requires special investigation, low platelet counts may be spurious, particularly due to the difficulty in collecting quantity and quality blood samples from neonates. This study was performed to determine the incidence of clumped or clotted blood in neonate CBC samples and variations in sample volumes and collection methods. Methods: Routine blood samples were collected from regular neonatal patients and neonatal intensive care unit (NICU). Sample collection used heel prick capillary blood, venous and arterial lines. Blood sample volumes were in 250 ul or 500 ul. Complete blood counts, including platelets, were determined using Sysmex automated analyzers. Each blood sample was examined for clots and platelet clump flag. Blood smears were prepared and reviewed for the presence of platelet clumps.

Results: A total of 1,040 neonatal samples were analyzed, 438 samples from regular neonatal units and 602 from NICU. 856 samples were collected by heel prick; 106 and 78 samples were collected by venous and arterial lines, respectively. A total of 130 samples, 12.5%, were found to have clot or showed platelet clump flag and platelet counts could not be determined. In addition, platelet clumps on blood smears were seen in 30% of the cases. No significant difference was seen between regular and NICU patients. Venous samples showed the least incidence of clotting and clumping, and a slightly higher incidence associated with 500 ul samples than 250 ul (13.9% vs. 12.1%).

Conclusions: A significant portion, 12.5%, of neonatal samples may give spurious low platelet counts due to clotting and clumping, and a higher incidence of platelet clumps was seen on blood smears even though no flags were shown on analyzers. Venous sampling method is a preferred method for neonatal blood samples. An increase in collection volume in 500 ul did not improve, but worsen the sample quality.
Introduction: Acquired hemophilia is a rare disorder due to autoantibodies inhibitors against factor VIII, mimicking the congenital hemophilia, often associated with autoimmune disease (mostly rheumatoid arthritis), malignancies, drugs, hematological or gastrointestinal disease. We report a case of acquired hemophilia associated with bullous pemphigoid, diagnosed only in second instance.

Methods: A 92-year-old woman was hospitalized with anemia, oral mucosa bleedings, hematuria and widespread ecchymoses. Medical history was silent for personal and family hemorrhagic manifestations or bleeding disorders and for assumption of drugs involving coagulation pathways. Blood tests: Hb 60 g/L, MCV 87 fL, aPTT 116 sec., aPTT Ratio 3.63, normal reference range for blood coagulation screening data (ACL Top 500). For persisting hemorrhagic manifestations, in the following days aPTT test mix and LAC were performed. LAC test was negative, but a strong mix test correction was observed (110 sec. premix/38 sec postmix.). According to these data, factor VIII and IX were tested. Results were respectively 0.5% and 97%. The titer of anti-factor VIII inhibitor Units was 4 Bethesda units (UB, normal value < 0.5). Results: The most likely diagnosis was of acquired hemophilia type A. Given the strong association of this condition with autoimmune diseases, diagnostic studies were carried out up to this diagnostic hypothesis. Because of the observation of bubbles in blood-serum content, dermatological advice was requested in the following days and a skin biopsy was performed. According to the tests results the diagnosis of bullous pemphigoid was done. The patient was administered recombinant factor VIIa, with clear improvement of hemorrhagic manifestations and blood crisis tests (aPTT 75 sec, aPTT RATIO 2.37, Hb 114 g/L). Currently, treatment is limited to steroid therapy, clinical conditions are steady and bleeding absent.

Conclusions: In elderly subjects, prolonged aPTT in the absence of other tested causes can be supported by an acquired defect of factor VIII (more rarely IX), when personal and family history is silent for previous hemorrhagic manifestations or bleeding disorders. This hypothesis should always be considered when an increased unmotivated aPTT is observed and the association with autoimmune diseases is always to be sought.

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605 SINGLE NUCLEOTIDE POLYMORPHISMS IN IMMUNE-REGULATORY GENES IN PATIENTS WITH IMMUNE THROMBOCYTOPENIA
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Introduction: Immune thrombocytopenia (ITP) is an autoimmune disease characterized by thrombocytopenia due to platelet autoantibodies, causing an accelerated clearance of opsonized platelets by phagocytes. The etiology of ITP remains unclear. Both genetic and environmental factors can have a role in the development of ITP. In this study, we investigated a possible association of some single nucleotide polymorphisms (SNP) in genes for interleukin beta (IL1β, -511C/T), tumor necrosis factor beta (TNFβ, +252G/A), tumor necrosis factor alpha (TNFα, -308G/A), cytotoxic T lymphocyte antigen 4 (CTLA4, +49 A/G), Fc gamma receptor (FCGR2A, H/R131 and FCGR3A, V/F158) with ITP.

Methods: We analyzed 125 adult ITP patients (35 men, 90 women) with median age of 47 (14-83) and 120 healthy matched controls. The median follow-up was 44 months (12-384). All patients were initially treated with corticosteroids, 38 were splenectomized. Forty two (34%) patients had refractory or unresponsive form of disease, according to the definition of the International Working group for ITP. Genotyping was performed by using polymerase chain reaction and restriction fragment length polymorphism method. The distribution of genotypes and allele frequencies were compared with a chi-squared test.

Results: Our results demonstrated significantly different genotype and allele distributions for TNFβ (+252 G/A) in patients with ITP (G/G=3, A/G=38, A/A=84) compared with controls (G/G=16, A/G=25, A/A=69), p=0.005 and p=0.009. We didn’t find significant differences in the genotype distribution or allele frequencies for TNFα (-308G/A) and ILβ (-511C/T), p=0.363 and p=0.845. There was significantly different genotype distribution and allele frequencies for TNFα (-308G/A) between patients with unresponsive and responsive ITP, P=0.016 and P=0.009. There was no significant difference in genotype distribution and allele frequencies for TNFβ (+252G/A) and ILβ (-511C/T) between these two subgroups of patients. Our results demonstrated significantly higher frequency of high affinity FCGR3A-158V allele in ITP patients compared with controls (47.2% versus 37.5%, p=0.037). We did not find significant differences in the allele frequencies for FCGR2A-131H/R, P=0.478. In the group of patients with unresponsive and responsive ITP, we found significantly different genotype distribution and allele frequencies for FCGR3A, P=0.036 and P=0.008 respectively. Our results confirmed that the combination of high affinity FCGR2A-131H and FCGR3A-158V allele was more common in ITP patients than in controls (55% versus 40%, p=0.024). Analysis of CTLA-4 polymorphisms did not confirm significant differences in genotype distribution between patients and controls, P=0.43.

Conclusions: Results of this study, suggest possible role of FCGR3A, TNF-β and TNF-α polymorphism in the etiology, development and clinical form of immune thrombocytopenia.

606 THE POTENTIAL OF ANTHOCYANIN-RICH QUEEN GARNET PLUM JUICE SUPPLEMENTATION IN ALLEVIATING THROMBOTIC RISK UNDER INDUCED OXIDATIVE STRESS CONDITIONS
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1Griffith University Southport, Australia, 2Central Queensland University Rockhampton, Australia, 3University of Tasmania Launceston, Australia

Introduction: Increased oxidant production in humans may induce a number of thrombotic consequences, including platelet hyperactivity/aggregability which could be countered through specifically developed functional foods. We compared the antithrombotic properties of anthocyanin-rich prune juice with prune juice supplementation with and without exercise-induced oxidative stress. Methods: Thirty three healthy participants were investigated in a randomized, double-blind, placebo-controlled, cross-over trial. Participants consumed 200 mL/day of prune, plum and placebo juice for 28 days, with treatments separated by a two-week wash-out period. Blood samples were collected pre and post supplementation as well as after 1 h of exercise (70% peak-O2 uptake) both before and after oral supplementation of plum juice and placebo. Platelet function and haemostatic activity were evaluated. Results: Only plum juice supplementation inhibited platelet aggregation induced by adenosine diphosphate (ADP) (< 5%, P = 0.02), collagen (< 2.7%, P < 0.001) and arachidonic acid (< 4%, P < 0.001); reduced platelet activation-dependent surface marker P-selectin expression of activated de-granulated platelets (< 17.2%, P = 0.04); prolonged activated partial thromboplastin clotting time (>2.1 s, P = 0.03); reduced plasma-fibrinogen (< 7.5%, P = 0.02) and malondialdehyde levels, a plasma biomarker of oxidative stress (P = 0.016). Plum juice supplementation also increased the anthocyanin metabolite plasma hippuric acid content (P = 0.018). Plum juice supplementation also inhibited ADP induced platelet aggregation both without and under exercise-induced oxidative stress by 10.7% (P < 0.01) and 12.7% (P < 0.001) respectively; arachidonic acid induced aggregation under oxidative stress by 28.8% (P < 0.05); reduced platelet activation-dependent P-selectin expression by 32.9% (P = 0.018). Plum juice supplementation reduced plasma-fibrinogen (< 7.5% (P < 0.001)) and arachidonic acid induced aggregation under oxidative stress by 28.8% (P < 0.05); reduced platelet activation-dependent P-selectin expression by 32.9% (P < 0.01) and 38.7% (P < 0.001) both without and under oxidative stress respectively; and exhibited favourable effects on coagulation parameters both with and without oxidative stress. Conclusions: Our findings suggest that plum juice but not prune juice has the potential to significantly attenuate thrombosis by reducing platelet activation/hyper-coagulability and oxidative stress. The anti-thrombotic activity exhibited by anthocyanin-rich plum juice suggests a potential for cardiovascular disease risk reduction and may be considered as complementary anti-platelet nutritional therapy in pro-thrombotic population.
EFFECTS OF IN VIVO AND EX VIVO P2Y12 ANTAGONISM ON PLATELET REACTIVITY FROM PATIENTS WHO UNDERWENT PERCUTANEOUS CORONARY INTERVENTION

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Introduction: Degree of platelet inhibition with antiplatelet drugs varies among individuals and inadequate levels are associated with adverse outcomes in patients. Herein, we tested the effects of in vivo and additional ex vivo antagonism on residual platelet reactivity.

Methods: Peripheral blood was collected from 443 patients who were on dual antiplatelet therapy with aspirin and clopidogrel and underwent percutaneous coronary intervention (PCI). Platelet reactivity was measured using VerifyNow® P2Y12 (PRU), light transmission aggregometry (LTA) with ADP (10 µM), and VASP (PRI) assays in the absence and presence of P2Y12 antagonist 2-methylthioadenosine 5’-monophosphate triethylammonium salt hydrate (2-MeSAMP) (30 and 100 µmol/L). Measures were analyzed as continuous and quartiles. Patients were categorized as poor responder (PR) to clopidogrel with PRU ≥240, LTA-ADP maximum ≥40%, or PRI ≥50%.

Results: Addition of 2-MeSAMP resulted in significant decreases in ex vivo platelet reactivity and number of PRs (Table 1 and Table 2). Data quartiles indicated that most patients stayed at the same quartile after addition of the antagonist. The 100 µM 2-MeSAMP caused 75-79% decrease in all quartiles of PRU. LTA-ADP maximum and PRI also showed decrease at all quartiles which was 47-67% with 30 µM 2-MeSAMP and 63-83% with 100 µM 2-MeSAMP. Possible pharmacokinetic influences of diabetes type-2, proton pump inhibitor (PPI) intake, and CYP2C19*2 variant on PR were also evaluated. The highest percentiles of patients with these conditions were localized in the 4th quartile of platelet reactivity measures. Addition of 2-MeSAMP resulted in similar decreases in platelet reactivity among the patients with and without these co-variants.

Conclusions: Degree of platelet response to clopidogrel decreased with the ex vivo addition of antagonist, consistent with a pharmacokinetic etiology of PR. This pharmacokinetic etiology is supported by the finding that patients with diabetes type-2, PPI use, and CYP2C19*2 variant were primarily in the 4th quartile of platelet reactivity.

Table 1

<table>
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<tr>
<th>Platelet reactivity measures</th>
<th>N</th>
<th>Platelet response to 2-MeSAMP (µM)</th>
<th>p-valuea</th>
<th>p-valueb</th>
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<tr>
<td>PRU</td>
<td>409</td>
<td>101 (77)</td>
<td>58 (41)</td>
<td>-</td>
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<td>LTA-ADP maximum (%)</td>
<td>443</td>
<td>27 (13)</td>
<td>14 (8)</td>
<td>10 (6)</td>
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<tr>
<td>PRI (%)</td>
<td>443</td>
<td>43 (24)</td>
<td>18 (12)</td>
<td>10 (12)</td>
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a = 0.05, b = 0.01

Table 2

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<th>Assay cut-off</th>
<th>N</th>
<th>Poor responder (PR) (N (%)</th>
<th>Prevalent PR (N (%))</th>
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<tr>
<td>PRU 2240</td>
<td>409</td>
<td>89 (22)</td>
<td>4 (1)</td>
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<tr>
<td>LTA-ADP maximum (%)</td>
<td>443</td>
<td>68 (15)</td>
<td>2 (0.3)</td>
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<tr>
<td>PRI 150%</td>
<td>443</td>
<td>189 (44)</td>
<td>24 (5)</td>
</tr>
</tbody>
</table>

PR, effect of addition of 2-MeSAMP

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IMMATURE PLATELET FRACTION IN PATIENTS WITH THROMBOCYTOPENIA USING SYSMEX XE-5000

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Introduction: Immature Platelet Fraction (IPF) is a marker of thrombopoietic activity in patients with thrombocytopenia. In order to distinguish normal or low thrombopoietic activity from increased thrombopoietic activity, a cut-off value of IPF is needed.

Methods: Subject of this study was 203 adults with thrombocytopenia (< 150,000/µL) and divided into 2 groups by clinician’s diagnosis. Group A is patients with normal (n=125) or low thrombopoietic activity and Group B is patients with increased thrombopoietic activity (n=78). All samples were analyzed using Sysmex XE-5000 after daily quality control performed.

Results: No subject with pseudothrombocytopenia was found in this study. Figure 1 shows that IPF value in thrombocytopenia with increased thrombopoietic activity is much higher than in thrombocytopenia with normal or low thrombopoietic activity. Optimal cut-off value of IPF was 7.65% with a sensitivity of 91%, specificity 92 %, positive predictive value 88%, negative predictive value 94%, and likelihood ratio of 11.38 times.

Conclusions: IPF cut-off value of 7.65 was able to distinguish thrombopoietic activity with excellent sensitivity and specificity.
700 CORRELATION OF LOW HB A2 WITH MOLECULAR STUDIES

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Introduction: Alpha thalassemia is one of the most common genetic disorders worldwide and results from decreased expression of one or more of the four alpha globin genes. Approximately 95% of alpha (a)thalassemia cases are due to gene deletions rather than point mutations. When evaluating patients with alpha thalassemia, four primary genotypes are possible: silent carrier (aa~/~a), a-thalassemia trait (~a/~a or ~/aa), HbH (b4) disease (~a/~a), and hydrops fetalis or Hb Barts (~~/~a). HbH disease (3-gene deletion) usually presents as moderately severe hemolytic anemia, while deletion of all 4 a-globin genes (Hb Barts) is incompatible with life. Carriers of the a-thalassemia trait have a mild hypochromic microcytic anemia, but couples with these genotypes can be at risk for having a fetus with hydrops fetalis or a child with HbH disease. Diagnosis of these cases is important for the genetic counseling purposes. Although decreased Hb A2 in Hb H disease has been described in the literature, its association with one or two gene deletion cases confirmed by molecular studies has not been well described.

Methods: We retrospectively evaluated 400 suspected cases of hemoglobinopathy referred to our laboratory for comprehensive evaluation. High-performance liquid chromatography analysis was performed using the manufacturer’s instructions for the Bio-Rad Variant II a-thalassemia Short Program (Bio-Rad, Hercules, CA), which separates hemoglobin variants by cation-exchange chromatography using a salt gradient. Our reference range for Hb A2 is 2-3.75%. Values less than or equal to 1.9% were designated as low Hb A2. Alpha-Globin gene deletions were detected using a previously published Gap–polymerase chain reaction (PCR) multiplex assay designed to detect the 7 most common deletions causing alpha-thalassemia. For alpha globin variants, bi-directional sequencing of the Alpha-Globin gene deletions were detected using a previously published Gap–polymerase chain reaction (PCR) multiplex assay designed to detect the 7 most common deletions causing alpha-thalassemia. For alpha globin variants, bi-directional sequencing of the

Effectiveness of HPLC when ordered.

Results: Of the 2444 samples tested, 42% (1079) had a haemoglobinopathy. The most common haemoglobinopathy was haemoglobin S, with 388 samples, followed by β-thalassemia minor, with 358. Related to haemoglobin variants, the most frequent was α-thalassemia (which is not detected by HPLC) followed by β-thalassemia. Conclusions: Due to the increased immigration during recent years in our country a sharp increase of haemoglobin S is observed overcoming β-thalassemia minor as the most prevalent haemoglobinopathy in our population. An accurate diagnosis of haemoglobinopathies is required to prevent inherited diseases and increase the effectiveness of HPLC when ordered.

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702 DEVELOPMENT, VALIDATION, AND IMPLEMENTATION OF A MULTIPLEX GAP PCR ASSAY FOR ALPHA THALASSEMIA DELETIONS USING FLUORESCENTLY LABELED CAPILLARY ELECTROPHORESIS
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Introduction: Alpha thalassemia, the most frequent hemoglobinopathy worldwide, is genetically heterogeneous, with most cases caused by variably sized deletions of the HBA1 and/or HBA2 loci. Deletions of HBA1 and HBA2 may be detected by a variety of methods, including Southern blot, multiplex ligation-dependent probe amplification, or gap polymerase chain reaction (PCR). A number of multiplexed gap PCR methods to detect the most common abnormalities, including –α-37, –α-42, –(α)3.7, –SEA, –MED, –THAI, and –THAI deletions, have been previously reported using gel based electrophoresis. In this study, we describe the development, validation, and implementation of a novel gap PCR procedure using two multiplexed, fluorescently labeled reactions and capillary electrophoresis for reliable, high throughput analysis in a clinical diagnostic setting. Methods: Initial attempts to utilize a single multiplex reaction for detection of all seven deletions failed to yield reproducible amplification. A revised protocol was created utilizing two multiplex reactions: 1) –α-37, –α-42, a “wild type” reference region of HBA2, and an amplification control; 2) –(α)3.7, –SEA, –MED, –THAI, and –THAI deletions, and an amplification control. PCR products from reaction 1 are digested with BstXI to produce smaller fragments for analysis. The resulting products from both reactions are subsequently pooled and analyzed by capillary electrophoresis (ABI 3730 genetic analyzer, Life Technologies, Grand Island, NY). Results: The assay was validated against a cohort of 36 samples previously shown at an outside laboratory to include –α-37, –α-42, –(α)3.7, –SEA, –MED, and –THAI deletions (n=6, 2, 2, 1, 6, and 8, respectively). Each of the 36 cases gave concordant results. Expected results were also obtained in 36 cases without any deletions. Conclusions: This novel multiplex gap PCR protocol reliably detects the seven most common deletions giving rise to alpha thalassemia. The use of fluorescently labeled capillary electrophoresis provides a high throughput workflow suitable to a clinical diagnostic laboratory serving a multi-ethnic population.

703 CELLAVISION DM96 DIGITAL RED CELL IMAGES SHOWING “FISH CELLS”: THEIR CONTRIBUTION TO THE &ALPHA; OR &BETA; THALASSEMIA DIAGNOSIS
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Introduction: Red blood cell (RBC) parameters are excellent for identifying microcytosis and hypocromia, but these findings are characteristics of both iron deficiency and thalassemia (T). Peripheral blood (PB) morphology in αT usually shows variable anisocytosis, poikilocytosis, microcytosis, hypochromia, tear drop cells, schistocytes and basophilic stippling. The objective was to explore the presence of fish cells in red cell images from PB using the Cellavision DM96 (DM96) analyzer in individuals who are homozygous or heterozygous for a or β T. Methods: We evaluated the presence of fish cells in PB red cell images in a total of 25 patients. From them, 14 patients were minor βT, 1 minor αT and 1 major βT. In addition, heterozygous α or βT was suspected in 9 patients in whom fish cells were detected. RBC parameters were obtained in the Advia 2120 analyzer. Morphological PB smear staining was done using May-Grünwald-Giemsa and red cell morphology was evaluated using the DM96 analyzer. High resolution HPLC was used for the quantitation of hemoglobins (Hb). Results: RBC counts showed mean values of 5.9 x 10¹²/L in the heterozygous patients, in which mean values of Hb were 119 g/L, hematocrit 39.0% and mean red cell volume 66.4 fl. All of these RBC parameters showed lower values in the major βT case. With respect to the RBC morphology analysis, digital images of RBC provided by the DM96 in the confirmed T patients showed not only the classic abnormalities described in these hemoglobinopathies, but also the presence of fish cells previously described by B Bain. Interestingly, in 9 of the patients included in this study the heterozygous form of T was suspected because of the characteristic RBC values and the presence of fish cells in the images. In 6 of them we found increased HbA2 levels (mean values 5.5%) and the remaining 3 patients showed low levels of HbA2 (1.9–2.1%) suggesting heterozygous forms of βT and αT respectively. Further molecular tests are in process to confirm the suspected diagnosis. Conclusions: Individuals heterozygous for α or β-thalassemia can be easily suspected by the RBC parameters and detection of fish cells in the digital images of the DM96. The observation of fish cells in the red cell morphology evaluation may help differentiate iron deficiency from thalassemia heterozygotes.
Molecular Screening of Thalassemia in Newborns in Northeast Thailand

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Introduction: Thalassemias are the major public health problems in Thailand and other Southeast Asian countries. Statistically in Thailand, the prevalence of α-thalassemia has been estimated to be 2.5-10% for α²-thalassemia, 1-8% for Hb Constant Spring and Hb Paksé and 15-20% for α²-thalassemia (−αα⁰ and −αα²) and that of 3-9% for β-thalassemia. These data are mostly determined using Hb analysis. In order to provide a more accurate information, we have re-examined the incidence of thalassemias using molecular techniques. Methods: Study was done on 350 newborn cord blood specimens. α- and β-thalassemia mutations were identified using the polymerase chain reaction (PCR)-based technologies and Hb analysis was performed using the Capillaries 2 Flex Piecing Hb analyzer (Sebia co.-, France). Genotypes were defined according to Hb and DNA results. Results: While twenty-three different genotypes were observed, no case with thalassemia major was encountered. The most prevalent one was Hb E (33.4%). The incidence of 3.1% α²-thalassemia (SEA deletion), 25.1% α²-thalassemia (3.7 kb deletion), 0.8% α²-thalassemia (4.2 kb deletion), 5.4% Hb Constant Spring and 1.4% of Hb Paksé were found. No α²-thalassemia with THAI deletion was found in this study. The incidence of β-thalassemia was found to be 0.6%. Hb capillary electrophoresis could demonstrate peaks of Hb E in all cases with Hb E carriers and homozygous Hb E, which could help in providing rapid diagnosis. Different levels of Hb Bart’s were detected for different α-thalassemia genotypes however Hb Bart’s was absent in some cases with one α-globin gene defect. Conclusions: Our results confirmed that α-thalassemia, β-thalassemia and Hb E are prevalence among the Northeast Thai population and complex interactions between them leading to several forms of thalassemia are common in the region. The prevention and control program launched 10 years ago to reduce number of new case with thalassemia major has been successful.

Hematology Analyzer Based Scoring System as Discriminating Guide for Iron Deficiency Anemia and Beta Thalassemia Minor

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Introduction: Iron deficiency anemia (IDA) and beta thalassemia minor (TM) are considered to be important cause of microcytic hypochromic anemia and requires serum iron studies and hemoglobin electrophoresis for confirmation. However, hematological parameters which are easily available on automated cell counters may be used for early differentiation of these two conditions. The present study was therefore conducted to analyze the diagnostic accuracy of established discriminative indices and to propose modified new score which may be used as an indicator of differentiation especially in regions where IDA predominates and may co-exist with TM. Methods: The study included all the cases with MCV < 80 fl and following serum ferritin and hemoglobin electrophoresis were divided into 4 groups, group I of IDA, group II of TM, group III of IDA with TM and group IV of suspected anemia of chronic disorder. Routinely available hematological parameters were analyzed to differentiate between these groups and to calculate the established ten discriminative indices. A new modified score of 11 was proposed by statistically analyzing previous discriminative indices with new cut off and giving score 0 for IDA and score 1 for TM. The summation of all scores gave modified 11 score which was statistically analyzed to discriminate IDA from TM. Results: Total 153 cases were included with group I including 64, group II 42, group III 18 and group IV 29 cases. Routine hematological parameter RDW, hematocrit and RBC count showed statistical significant difference between group I and II, MCHC and MCH between group II and III and RBC count between group I and group III (p< 0.05). Platelet count and PDW was significantly more in IDA while MPV was more in TM. The proposed modified 11 score showed sensitivity and specificity of 87.69% for IDA and TM respectively and area under the ROC curve was 0.735. The positive predictive value for TM was 76.47% and for IDA was 77.03% with Youden index of 48.15 and percentage of correctly identified patient as 76.85%. Conclusions: The proposed new modified 11 score which is easily calculated by routinely available hematological parameters may be used for initial differentiation of IDA from TM particularly in regions where iron deficiency predominates. It is essential for every laboratory to define its own cut off for adequately using the score as differentiating indicator. This initial guidance may limit the use of advanced confirmatory techniques especially in resource limited settings.
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RETICULOCYTE PARAMETERS IN THE DIFFERENTIAL DIAGNOSIS BETWEEN IRON DEFICIENCY ANEMIA AND BETA-TALASSEMIA
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Introduction: Microcytosis and hypochromia are the main red blood cell (RBC) features that iron deficiency anemia (IDA) and beta-thalassemia (BT) have in common. Since the Seventies numerous formulas have been published with the intention of assisting a screening algorithm among those entities. In recent years technological advances have been introduced into hematology analyzers providing new parameters related to RBC population. This additional information can improve diagnostic specificity. Some of these parameters are related to reticulocytes, including reticulocyte hemoglobin equivalent (Ret-He) and reticulocyte maturity indices (low, medium and high fluorescence ratio (LFR, MFR and HFR respectively) or percentage of fragmented red cells (FRC). The last four parameters require reference values to be used in the clinical practice. The aim of the study was to assess the diagnostic value of classic and research parameters of RBC in the screening of IDA and BT, and define the most accurate formula for our given population. Methods: 127 patients (68% women) were included: 59 with IDA and 69 with BT. Samples were analyzed at Sysmex –XE 2100. Evaluated parameters were RBC, Hb, MCV, MCHC, RDW, total reticulocytes number (RET), Ret-He, HFR, MFR, LFR and FRC. Different formulas were studied and all of them included FRC in their equation. ROC curve analysis was performed to determine diagnostic accuracy. Results: Most of red cell parameters analyzed showed statistically significant difference between IDA and BT (Table 1). The FRC was the single parameter with better discriminant efficiency (AUC: 0.824, IC: 0.751-0.898; p< 0.001). The combination between classical and novel parameters provided several formulas with different AUC (Table 2). The formula (RDW/RBC)xFRC was the most efficient to distinguish IDA from BT (AUC: 0.919, IC95%: 0.867-0.971; p< 0.001), and the best cut-off was 8.3 (SE=91.5%, SP=84.8%). Conclusions: FRC parameter and its related formula (RDW/RBC) x FRC have a good discrimination capacity for the differential diagnosis between IDA and BT in our population. This can improve laboratory efficiency since the addition of tests for a definitive diagnosis can be more cost-effective.

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TWO INDEPENDENT ORIGINS OF B’-THALASSEMIA DUE TO -31 A TO G MUTATION
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Introduction: A single base substitution (A-G) at nucleotide -31 within the TATA box of b-globin gene has been described originally in Japanese patients with b-thalassemia. It has been proposed that this mutation arose primarily in Japanese. We have found that this mutation is the second most common b-thalassemia allele among northeast Thai population. In order to address on the origin of this b-thalassemia gene in Thai population, we have examined b-globin gene haplotype associated with this mutation and compared with that described for Japanese subjects. Methods: Seven polymorphic sites within b-globin gene cluster (3'-Hinc I, g- & g'-Hind III, yb- & 3'yb-Hinc II, b-Jva II and b-Hinfl) were determined using allele specific PCR methods newly developed for rapid b-globin haplotyping. These seven DNA polymorphisms could be examined on 3 multiplex and a single monoplex PCR formats without restriction digestion. Subjects included 7 b-thalassemia carriers with -31 A-G mutation. Associated haplotype with the mutation was defined. Results: A concordant result of DNA polymorphisms examined using allele specific PCR assays and conventional PCR-RFLP methods were observed. It was found that this Thai b-thalassemia mutation was associated with b-globin gene haplotype (+- - - - - - +) which is different from that described for Japanese subjects; (-+ - - + + -). Conclusions: Apparently, two independent origins of the -31 (A-G) b-thalassemia gene were noted, one in Japanese and another in Thai. This mutation is the most common b-thalassemia gene among Japanese and is relatively common among Thai population. As compared to the PCR-RFLP assay, the b-globin haplotyping assays developed in this study is easier, rapid, lesstime consuming and requires no restriction digestion. Therefore, the methods should prove useful in population genetic study of b-hemoglobinopathy.
META-ANALYSIS OF DISCRIMINANT FUNCTIONS FOR DIFFERENTIATING THALASSEMA FROM IRON DEFICIENCY ANEMIA

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Introduction: More than 40 mathematical functions have been proposed in the hematological literature for discriminating between iron deficiency anemia (IDA) and thalassemia (thal) trait in subjects with microcytic red blood cells (RBC). None of these discriminant functions (DF) is 100% sensitive and specific and comparative studies have shown that even the ranking of the DF’s is not consistent between the various studies. As most studies are of limited size, we decided to conduct the first meta-analysis of the most frequently used DF’s. Methods: An extensive literature search yielded 147 published studies that reported the diagnostic performance of one or more DF. For obtaining sufficient statistical power, we selected only the 99 articles dealing with those 11 DF’s that have been investigated five or more times. For each DF we calculated the diagnostic odds ratio (DOR) as the main performance indicator. Results: The microcytic/hypochromic RBC (M/H) ratio showed the best performance, with DOR=115 (95% confidence interval: 49-270). This DOR was significantly higher than that of all other indices investigated. The Sirdah index scored second with DOR=47 (CI: 25-88), closely followed by the Ehsani index with DOR=42 (CI: 26-67). Subsequently, there was a group of four indices with DOR between 25 and 30: England & Fraser, Green & King, RDW Index (Jayabose) and Mentzer index. The Ricerca, Srivastava and Shire & Lal indices had a low DOR (around 14), and the lowest performance (DOR=6; CI: 4-10) was found for the RDW (Bessman index). Overall, the indices performed better for adults than for children. Studies in European countries demonstrated higher DOR values than in Mediterranean and Southeast Asian populations. The influence of hematology analyzers on DF outcome was negligible. Conclusions: The M/H ratio outperformed all other discriminant functions for discriminating between IDA and thal trait. Although its sensitivity and specificity are not high enough for making a definitive diagnosis, it is certainly of value for identifying those subjects with microcytic RBC in whom confirmatory diagnostic tests are warranted.

THE IMPORTANCE OF NUCLEATED RED BLOOD CELLS IN PATIENTS WITH BETA THALASSEMA MAJOR AND COMPARISON OF TWO AUTOMATED SYSTEMS WITH MANUAL MICROSCOPY AND FLOW CYTOMETRY

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Introduction: Nucleated red blood cells (NRBC) can be used as a marker of erythropoietic stress and can help optimize transfusion therapy in patients with beta thalassemia major. The aim of this study was to determine this correlation; and compare two automated systems (Sysmex XE-2100, and Advia 2120i) with manual microscopy and flow cytometry (FCM). Methods: Absolute NRBC counts and percentages (NRBC%) from 51 patients analyzed with both automated and reference methods. The results were compared with levels of pre-transfusion hemoglobin and ferritin levels. Results: The mean age of the included patients (31 female, 20 male) were 12.9±7.5 years. Mean levels of hemoglobin and ferritin were 9.5±1.2 g/dL and 1896±1194 ng/mL respectively. The NRBC% of two instruments did not significantly differ and correlated well (p<0.0001, r=0.984). NRBC% with XE-2100 and Advia 2120i versus with manual microscopy yielded also high correlations (r=0.951 and r=0.981, respectively), however absolute NRBC counts versus FCM yielded lower coefficients (r²=0.723 and r²=0.694, respectively). High serum ferritin levels were correlated with both NRBC% and counts with both instruments (p<0.001) and hemoglobin levels lower than 9.0 g/dL had a negative correlation with NRBC% and NRBC counts with Advia 2120i (p<0.05, r=-0.495) but not with XE-2100. Conclusions: In this study for the first time the positive correlation between high ferritin and NRBC levels and a negative correlation with Hb was shown. Monitoring of NRBC% with both instruments can help optimizing transfusion therapy for patients with beta thalassemia major. NRBC can be used for a more effective control of transfusion therapy in transfusion-dependent thalassemic patients.
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CHANGE OF RBC DEFORMABILITY DURING HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Introduction: The red blood cell (RBC) deformability test is the measurement of the ability of the RBCs to adapt their shape to the flow conditions. The major determinants of the deformability include cell shape, composition of the cell membrane and its cytoskeleton, and internal viscosity (mean cell hemoglobin concentration). The contribution of the erythrocyte membrane to the deformability is primarily regulated by the composition and arrangement of its structural constituents. In cancer patient, chemotherapy and hematopoietic stem transplantation (HSCT) change bone marrow microenvironment, these may affect the RBC production and deformability. Thus we evaluate the change of RBC deformability during HSCT. Methods: Blood samples were obtained from each patients who underwent HSCT. Red cell deformability was measured with a microfluidic ektacytometer (RheoScan-D, RheoMeditech, Seoul, Korea). All analyses were completed within 24 hours after blood collection. The elongation index (EI) of erythrocytes is defined as (L−W)/(L+W), where L and W are the major and minor axes of the ellipse, respectively. Results: Eleven children (3 acute lymphoblastic leukemia, 5 acute myeloid leukemia, 1 aplastic anemia, 1 Ewing sarcoma, 1 retinoblastoma) were enrolled in this study. The EI of RBCs were decreased to D+5 from D-day and slowly increased from D+5 to D+30 after hematopoietic stem cell transplantation. Conclusions: The deformabilities of RBCs were slowly increased during hematopoietic stem cell transplantation. RBC deformability may reflect bone marrow microenvironment of patient during HSCT. Further study about correlation with RBC deformability and prognosis of HSCT is needed.

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HEMOGLOBIN VARIANTS IN NORTHERN THAILAND: MOLECULAR AND HEMATOLOGICAL CHARACTERISTICS

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Introduction: Hemoglobin variant is an inherited disorder that results in the abnormal structure of globin chains of the Hb molecule. Although, most of them are not pathologically relevant, coinheritance of a- and b-thalassemias may lead to clinical condition and difficulty in laboratory diagnostics. In Thailand, the prevention of control program of thalassemia has been implemented all over the country, number of samples subjected to Hb analysis have been increasing. Thus, number of abnormal Hbs is increasingly encountered. However, most of them could not be diagnosed during routine Hb analysis and further investigation based on DNA testing is usually required. The molecular information of Hb variants will facilitate the prevention and control program and genetic counseling. Methods: Two hundred and eleven blood samples of the subjects with suspected for abnormal Hbs during routine Hb analysis were obtained from several hospitals in 8 provinces of upper northern Thailand during June 2012 – January 2014. The corresponding mutations on a-, b- and d-globin genes were identified using allele specific PCR and DNA sequencing. Results: Hb and DNA analyses identified altogether 14 different abnormal Hbs. Five a-chain variants including Hb Q-Thailand, Hb Hekinan, Hb Siam, Hb Beijing and Hb Kawachi were detected in 40 (19.0%), 8 (3.8%), 2 (0.9%), 1 (0.5%) and 1 (0.5%) cases, respectively. Seven b-chain variants including Hb Hope, Hb Tak, Hb J-Bangkok, Hb S, Hb G-Makassar, Hb Korle-Bu and Hb C were observed in 115 (54.5%), 30 (14.2%), 3 (1.4%), 3 (1.4%), 1 (0.5%), 1 (0.5%) and 1 (0.5%) cases, respectively. Two d-chain variants were identified in 5 subjects comprising of 4 (1.9%) subjects with Hb Aγ-Melbourne (d^{E47AG-52Ac}) and one with a novel-chain variant, namely the Hb Aγ-Melbourne (d^{E47AT-52Ac}). Several genetic interactions between these Hb variants with thalassemias were noted and associated hematological phenotypes were recorded. Conclusions: The results indicate the genetic heterogeneity of abnormal Hb among the upper northern Thai population which is difference from those reported in other parts of the country. Basic information obtained in this study should prove useful in laboratory diagnostics of Hb variants and genetic counseling as well as further population genetic study of hemoglobinopathies in the region.
IDENTIFICATION OF THE HEREDITARY SPHEROCYTOsis: NEW OPTIONS OF LABORATORY DIAGNOSIS.

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Introduction: Hereditary spherocytosis is common erythrocyte membranopathy. Frequency of occurrence of the disease makes 1 case per 2-5 newborns. Hereditary spherocytosis often causes a complex aggregate of clinical signs, hemolytic crises in patients. At the same time many patients have asymptomatic hereditary spherocytosis. Differential diagnosis of hereditary spherocytosis is quite complex and in modern workload conditions the clinical doctors need a simpler diagnostics procedure. Methods: In this study we have assessed diagnostic value of hematological parameters provided by the hematological analyzer Beckman Coulter Cellular Analysis System DxH800 for identifying the degree of erythropoiesis disorder in patients with hereditary spherocytosis at the stage of maturing of reticulocytes. An assessment of significance of estimate indicators RET/IRF, MCV-MSCV was carried out for primary diagnostics of hereditary spherocytosis. The diagnosis of hereditary spherocytosis was confirmed by means of cosin-5 maleimide binding test (flow cytometry), Deich`s method of determination of erythrocyte osmotic resistance and method of electrophoresis of erythrocyte membrane protein in polyacrylamide gel using Lemley technique. Participants included 317 adults with no hematological disorders, 42 children with no hematological disorders, 13 adults with verified hereditary spherocytosis and 43 children with identified hereditary spherocytosis. Results: Evaluation of the erythrocytes and new reticulocytes parameters at the hematological analyzer identified the significant difference in estimate index MCV-MSCV (p< 0.0001) and RET/IRF (p< 0.0001) between group of patients with hereditary spherocytosis and control group. The cut level of delta MCV-MSCV> 11 for the positive hereditary spherocytosis cases, medium value for this group = 24.5 (for the control group medium value of delta MCV-MSCV= 6.2). Area under ROC-curve = 1,0; specificity=100%; sensitivity =100%. The medium value of index RET/IRF for patients with hereditary spherocytosis = 19,5 ( for the control group medium mean of RET/IRF=3,6. The cut level of RET/IRF for hereditary spherocytosis positive cases= 6,9 (area under ROC curve = 0,97, specificity = 94%; sensitivity =96,3%). In this study we also found significant difference of classic erythrocyte indexes RBC, HgB, MCHC, RDW in patients with hereditary spherocytosis comparing to this indexes for control groups (changes of this indexes are not specific for hereditary spherocytosis). Conclusions: According to our data, research with the hematological analyzer could be recommended as the screening option for the first identification of hereditary spherocytosis in patients and determine the estimated parameters for the values of the patients MCV-MSCV and RET/IRF.

HB HASHARON AND HB O-INDONESIA LOCATED ON OPPOSITE ALPHA GENES

Toon Schiemsky1, Cornelius Hartevedt2, Koen Desmet1, Davy Kieffer2
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Introduction: We report two cases in which hemoglobinopathy screening warranted DNA analysis. This lead to the incidental finding of 2 known hemoglobin (Hb) mutations located on the opposite alpha gene as yet described. Methods: Results: In the first patient, a Belgian man of 66 years old, hemoglobinopathy screening was triggered due to an aberrant HbA2c (CE-HPLC) analysis. Furthermore, the presence of microcytic red blood cells was noted. Further investigation using CE-HPLC (Bio-Rad, VARIANT™ I β-Thalassemia Short Program) depicted an extra fraction of 26.5% (rt 4.77min), which presence was confirmed using alkaline and acid gel electrophoresis (Hydragel (acid) 7 Hemoglobin, Sebia, Cedex, France). HbA2 was low (1.2%). DNA analysis was performed (Hemoglobinopathies Laboratory, LUMC, The Netherlands) and revealed a heterozygous –alpha3.7 (Rightward) deletion and a heterozygous HBA1c.142G>C mutation in the HBA1 gene. This patient is carrier of mild alpha thalassemia and Hb Hasharon. However, this mutation has not yet been described in the HBA1 gene. The observed variant fraction is higher than described (14-19%, HbVar database) and can probably ascribed to the co-existing mild α-thalassemia. This variant has no clinical implications. The second patient is a 34 year old African woman, presenting with increasing fatigue and exercise intolerance. A microcytic, hypochromic anemia and low iron status were found. However, considering the African descent and to exclude the presence of HbS, hemoglobinopathy screening using CE-HPLC was performed revealing an extra fraction of 9.7% (rt 4.88 min). HbA2 was low (2.1%). The presence of the extra fraction was confirmed using gel electrophoresis. Molecular analysis showed a HBA2c.349G>A mutation, known as Hb O-Indonesia. Again, this variant has only been described in the HBA1 gene, not the HBA2 gene. The variant fraction is lower than described in literature (21%, HbVar database). Since no arguments for an α- or β-thalassemia were found, this can probably be explained by the low iron status. Conclusions: We report two cases in which hemoglobinopathy investigation led to the detection of two known mutations occurring in the opposite alpha genes than those described in current literature. Furthermore, the detected fractions of the variants and the phenotypical characteristics of the patients did not correspond with those described in the literature, respectively due to an underlying α-thalassemia and iron depletion.
MULTIPLE DISCRIMINANT ANALYSIS FOR ALPHA THALASSEMIA SCREENING
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Introduction: Various indices have been defined to discriminate iron deficiency anemia and β-thalassemia, based on red cell parameters, but no specific index has been defined for α-thalassemia screening. Powerful statistical computer programming enables sensitive discriminant analyses to aid in the diagnosis. We investigated the capabilities of the multiple discriminant analysis (MDA) for the differential diagnosis of microcytic anemia, genetic or acquired. The aim of the present study was to investigate the performance of standard indices and MDA for detecting α-thalassemia carriers.

Methods: The training group was composed of 170 subjects with iron deficiency anemia (IDA), 90 α and 200 β-thalassemia carriers. A set of potential predictor parameters that could detect differences among microcytic were selected: Red Blood Cells (RBC), hemoglobin (Hb), mean cell volume (MCV), mean cell hemoglobin (MCH) and RBC distribution width (RDW). The obtained functions were applied to a series of patients with microcytic anemia extracted from the Laboratory workload: 116 IDA, 97 α and 200 β-thalassemia carriers (learning group). Receiving operator curves analysis was used to compare the performances of equations and indices.

Results: AUC obtained in the ROC analysis for indices: Bessman 0.721, England & Frazer 0.884, Eshani 0.874, Green & King 0.899, HH 0.798, Mentzer 0.868, MHRatio 0.788, RDW-I 0.893, Ricerca 0.836, Shine & Lal 0.704, Sirdah 0.898, Srivastava 0.822 MDA rendered two linear equations: Thal Index, for beta and alpha carriers detection (Anemia doi 10.1155/2013/457834) RBC 1.778 Hb -2.225 MCV 1.814 MCH 0.152 RDW 0.441 ROC results AUC 0.960 cut off 124.0 sensitivity 91.3 % specificity 91.4 % Alpha Hb -2.225 MCV 1.814 MCH 0.152 RDW 0.441 ROC results AUC 0.954 cut off 38.1 sensitivity 91.7 % specificity 90.5 %

Conclusions: Linear discriminant functions based on routine hemogram data can effectively differentiate between genetic and acquired anemia; for α-thalassemia carriers the performance is better than those of standard indices so samples can be efficiently selected for further molecular analysis to confirm the presence of thalassemia.

THE DIAGNOSTIC PERFORMANCE OF RED CELL COUNT FORMULAE IN EVALUATION OF MICROCYTOSIS REGARDED TO BETA THALASSEMIA TRAIT
Galina Zemtsovskaja, Marika Pikta
North Estonia Medical Centre Tallinn, Estonia

Introduction: The main conditions that lead to microcytosis are iron deficiency anaemia and thalassemia. Microcytosis in thalassemia appears due to reduction in the amount of normal globin chains. In case of reduction of beta-globin chains and subsequent excess of alpha-globin chains the production of one of the normal hemoglobin A2 (HbA2) is increased. Corresponding clinical condition is named as beta-thalassemia and formed heterozygote condition defined as beta-thalassemia trait (BTT). Measurement of HbA2 concentration is important in routine diagnosis of BTT and DNA analysis may be required in cases of doubt due to findings of atypical hemoglobin. The role of extended measurement of red blood cell (RBC) subpopulations and their count-derived formulae were investigated by various researchers during the last few years. The aim of our investigation was to assess the diagnostic performance of red cell count (RCC)-based formulae derived from the percentages of microcytic and hypochromic RBC among microcytic samples using elevated HbA2 concentration for diagnosis of BTT.

Methods: Data of 170 investigated samples using elevated HbA2 concentrations above 3.5% was used for the North Estonia Medical Centre laboratory due to a microcytosis (mean cell volume, MCV < 80fL) and suspicion of BTT was investigated. The sensitivity, specificity, positive, negative predictive values (PPV and NPV) and efficiency of %microcytic-%hypochromic RBC (cut-off>11,5%) and %microcytic-%hypochromic RBC-RDW (cut-off>5,1%) indexes were assessed in regards to BTT-diagnostically elevated HbA2. Cut-off above 3.5% was used for HbA2 and statistical analysis was performed with Microsoft Excel. Results: Data of 27 investigated samples of adolescents and adults (age ranges 11-70, median 38,5) with HbA2 concentrations above 3,5% (median 15,7, range 4,6% – 6,1%, 12 samples with genetically confirmed BTT) and 32 samples (age 12-73, median 44) with HbA2 ≤3,5% (median 2,2%, range 1,6%-3,2%) were included in the assessment. One sample with HbS (borderline HbA2 of 3,7%) was excluded. We had no confirmation of the status of iron metabolism for most samples. The sensitivity, specificity, PPV, NPV and efficiency for %microcytic-%hypochromic index were 85, 88, 85, 88, 93% and for %microcytic-%hypochromic-RDW -- 93, 88, 86,93 and 96%, respectively.

Conclusions: The performance of both indexes was sufficiently good. %microcytic-%hypochromic-RDW index showed the better possibility for exclusion of BTT and the better efficiency in correct categorization of all samples.
Introduction: Red Blood Cells (RBC) ability to withstand mechanical stress without hemolysis is critical to their in vivo survival and oxygen delivery. RBC membranes can be compromised by pathology, procedures, storage, or by blood-contacting devices or drugs. The impact on RBC is typically evaluated through hemolysis; this however ignores sub-hemolytic cell damage that can result in subsequent hemolysis in vivo. Testing for RBC Mechanical Fragility (MF) is recognized as the best way to assess sub-hemolytic cell damage, however the test's use is hindered by the lack of a convenient and standardizable testing system. Here we present an approach to making the testing integrated and automated, and thus also conducive to comprehensive RBC MF profiling with multiple indices. Such a system could be suitable for a variety of research, development, and clinical applications. Methods: MF profiles of RBC each depicted fractional hemolysis upon incremental durations of applied mechanical stress. Stress was applied via a customized bead mill with oscillation frequencies between 3 and 50 Hz at durations from 15s to 30m. Optical analysis was done using a proprietary approach that allows determining hemolysis without a need for RBC separation. Results: Both human and bovine RBC exhibited significant individual variability in MF. Such differences were not correlated to pre-existing hemolysis. While dilution with normal saline can increase MF, plasmaphoresis with plasma replacement by albumin-supplemented media in a patient did not result in alteration of original MF. Changes in MF due to various drugs was observed – e.g. hydroxyurea in sickle cell patients, for which MF testing offers the potential to monitor treatment. Certain stress intensities and/or types had qualitatively different influences on results, allowing for selection of stresses to target particular needs and applications. For example, in assessing stored blood, “low-energy” stress was found to give MF results more relevant to RBC changes versus “high-energy” stress. MF was found to significantly depend on the RBC chemical environment, with albumin increasing cells' ability to withstand mechanical stress. Profiles in blood plasma were found to depend on the presence of fatty-acids, with plasma lipid load acting to diminish protective effects of the protein. Conclusions: The presented approach for RBC MF testing can offer an easy and standardizable way to assess sub-hemolytic blood damage for a range of applications in academia and industry.
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THE USE OF INTERNAL QUALITY CONTROL FOR ERYTHROCYTE SEDIMENTATION RATE (ESR)
Gail Earl, Katherine Marsden, John Sioufi
RCPAQAP Pty Limited Sydney, Australia

Introduction: An external quality assurance program for the Erythrocyte Sedimentation Rate (ESR) has been available as an RCPAQAP Haematology module since 2008. The results returned by participating laboratories for this module have shown wide variation and questionnaires have been used to try to determine a cause, e.g. method of handling the survey material. The latest questionnaire was distributed to ascertain the type of internal quality control (IQC) performed by participating laboratories and whether this could contribute to the result variation. We also aimed to determine the procedures for corrective actions in response to non-conforming survey results. Methods: A questionnaire was distributed to 483 laboratories enrolled in the RCPAQAP Haematology - ESR module. Questions pertained to the use of internal quality control for ESR testing, the type of laboratory, ESR methods, type of controls and how laboratories assessed acceptable performance. Results: Responses were received from 254 of the 483 laboratories. A wide variety of practices are used as IQC for ESR. It was more frequently performed for automated methods (>85% of respondents) than manual methods (< 50%). The frequency varied being daily in the majority, as well as 2-3 times weekly, monthly, with every batch of ESRs, with each new box of pipettes or with each batch of diluent. Commercial material was used in >90% of laboratories performing IQC and 7% used patient samples. Interlaboratory comparative exercises and statistical analysis of patient data were also reported. Most respondents reviewed their EQA data in conjunction with their internal QC. Conclusions: Most respondents participate in IQC, some implementing creative solutions to overcome technical difficulties in their lab. The wide variety of IQC practices may contribute to variation of ESR results. The RCPAQAP Haematology cannot mandate internal QC testing of ESRs, however labs are encouraged to develop a program suitable to their situation.

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REGULATORY CONSIDERATIONS FOR THE CLEARANCE OF HEMATOLOGY IN VITRO DIAGNOSTIC DEVICES
Niquiche Guity
Hematology Branch, Division of Immunology and Hematology Devices, Office of In Vitro Devices, Food and Drug Administration Silver Spring, MD, USA

Introduction: The mission of the Center for Devices and Radiological Health (CDRH) is to protect and promote the public health. We assure that patients and providers have timely and continued access to safe, effective, and high-quality medical devices and safe radiation-emitting products. The regulatory review process for in vitro diagnostic devices can seem overwhelming and cumbersome, but with some guidance from the Food and Drug Administration (FDA) this process can proceed smoothly. This presentation will provide an overview of the 510(k) process, outlining areas in which it could be helpful to interact with the agency. To this end, a summary of the different interaction types will be provided. We will further discuss device and product classifications, devices that are appropriate for the 510(k) program, as well as the content of the 510(k) and study design considerations. Methods: Using FDA guidance documents and online resources, an overview of the 510(k) process will be provided. Study design considerations for clinical and analytical testing will be outlined using Clinical and Laboratory Standards Institute (CLSI) documents. Results: We list major agency interactions prior and during 510(k) submission, designed to facilitate the successful completion of the regulatory clearance process. Additionally, general considerations for clinical and analytical study design will be presented. Conclusions: This presentation is to provide a better understanding of the possible interactions with the FDA, and provide recommendations for 510(k) study design applicable to hematology in vitro diagnostic devices.
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SETTING APPROPRIATE PRECISION LIMITS IN ROUTINE HEMATOLOGY

Anna Johnston¹, Andrew McFarlane¹, Gini Bournier², Tracy Martin³, Berna Aslan¹, Ruth Padmore¹
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Introduction: Automated hematology analyzers have evolved to improve workflow and precision. Precision, defined as the closeness of agreement between replicate measurements, is expressed quantitatively in terms of imprecision either as the Standard Deviation (SD) or the coefficient of variation (CV). Results from an Institute for Quality Management in Healthcare (IQMH) patterns-of-practice survey demonstrated considerable variation in precision goals reported by laboratories. A literature review provided limited guidance in setting precision goals for analytical performance of hematology analyzers. The IQMH Hematology Committee developed precision goals to provide guidance for laboratories in setting appropriate precision limits for routine hematology parameters.

Methods: Current published literature for setting precision goals was reviewed along with those reported from a patterns-of-practice survey of hematology laboratories, claims from various hematology analyzer manufacturers, and members of the IQMH Hematology Committee. These were compared to the CV (%) and SD of 201 CBC results reported from a recent IQMH proficiency testing survey grouped by hematology instruments. The IQMH Allowable Performance Limits (APLs) represents the total allowable error of agreement between replicate measurements, is expressed quantitatively in terms of imprecision either as the Standard Deviation (SD) or the coefficient of variation (CV). Results from an Institute for Quality Management in Healthcare (IQMH) patterns-of-practice survey demonstrated considerable variation in precision goals reported by laboratories. A literature review provided limited guidance in setting precision goals for analytical performance of hematology analyzers. The IQMH Hematology Committee developed precision goals to provide guidance for laboratories in setting appropriate precision limits for routine hematology parameters.

Conclusions: Precision goals are an important consideration when selecting and validating new instruments. Setting appropriate precision limits are useful for monitoring QC and for ongoing analytical performance of established methods. Our results showed that setting a precision equivalent to one-third of the APLs could be useful for laboratories.

Table 1: Routine IQMH Hematology Precision Limits and APLs

<table>
<thead>
<tr>
<th>Test</th>
<th>Concentration</th>
<th>Precision</th>
<th>APL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukocyte Count</td>
<td>&lt; 5.0 x 10^9/L ≥ 5.0 x 10^9/L</td>
<td>± 0.2 x 10^9/L ± 3%</td>
<td>± 0.5 x 10^9/L ± 10%</td>
</tr>
<tr>
<td>Erythrocyte Count</td>
<td>&lt; 2.0 x 10^12/L ≥ 2.0 x 10^12/L</td>
<td>± 2.0 x 10^12/L ± 5%</td>
<td>± 0.2 x 10^12/L ± 5%</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>&lt; 100 g/L ≥ 100 g/L</td>
<td>± 2.0 g/L ± 2%</td>
<td>± 4.0 g/L ± 5%</td>
</tr>
<tr>
<td>Hematocrit</td>
<td>&lt; 0.20 L/L ≥ 0.20 L/L</td>
<td>± 0.01 L/L ± 2.5%</td>
<td>± 0.02 L/L ± 5%</td>
</tr>
<tr>
<td>Mean Cell Volume</td>
<td>&lt; 50 fl ≥ 50 fl</td>
<td>± 1 fl ± 1.5%</td>
<td>± 5 fl ± 5%</td>
</tr>
<tr>
<td>Platelet Count</td>
<td>&lt; 20 x 10^12/L ≥ 20 x 10^12/L</td>
<td>± 2.0 x 10^12/L ± 5%</td>
<td>± 1.0 x 10^12/L ± 15%</td>
</tr>
<tr>
<td>Mean Platelet Volume</td>
<td>All</td>
<td>4%</td>
<td>± 12%</td>
</tr>
<tr>
<td>Granulocyte, Lymphocyte, Monocyte, Eosinophil and Basophil Counts</td>
<td>&lt; 4.0 x 10^9/L ≥ 4.0 x 10^9/L</td>
<td>± 0.2 x 10^9/L ± 3%</td>
<td>± 0.5 x 10^9/L ± 15%</td>
</tr>
</tbody>
</table>

Conclusions: Precision goals are an important consideration when selecting and validating new instruments. Setting appropriate precision limits are useful for monitoring QC and for ongoing analytical performance of established methods. Our results showed that setting a precision equivalent to one-third of the APLs could be useful for laboratories.

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A COMPARISON OF THE QUALITY OF CRYOPRESERVED UMBILICAL CORD BLOOD USING THE AUTOMATED BIOARCHIVE SYSTEM AND THE CONVENTIONAL FREEZER

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Introduction: Successful engraftment in hematopoietic transplantation depends on the dose and quality of cells in the umbilical cord blood (UCB). To improve the quality of UCB, various cryopreserved methods have been developed at UCB banking systems. After processing, UCB is usually placed in the control rate freezer, is frozen at a programmed rate, and is then transferred to a liquid nitrogen storage container (i.e., the conventional method). The automated system (BioArchive®, Thermogenesis, Sacramento, CA, USA), which combines a controlled freezing rate with storage in the same device, was developed to decrease transient warming events. In this study, we compared the automated and conventional methods by assessing the quality of the UCB unit.

Methods: From April 2013 to October 2014, 90 UCB units were obtained from the CHA Medical Center Cord Blood Bank. We evaluated 50 UCB units that were cryopreserved using the conventional method and 40 UCB units that were cryopreserved using the automated system. After thawing the UCB, the total nucleated cells (TNCs) and CD34+ cell counts, cell viability (defined by trypan blue and 7-amino actinomycin D [7-AAD] staining), and colony-forming unit-granulocyte/macrophage (CFU-GM) were analyzed.

Results: There were no significant differences in the maternal and neonatal factors and pre-freezing variables in the UCB between the two methods. The mean recovery rates of the TNCs and CD34+ cells were 83.8% ± 6.9% and 103.2% ± 40.5%, respectively, using the conventional method and were 83.8% ± 7.6% and 108.1% ± 52.8%, respectively, using the automated method. The cell viability and progenitor cell content in post-thawing UCB units are shown in Table 1. There were significant differences in the CD34+ cell viability and CFU-GM content between the methods.

Table 1: Comparison of cell viability and progenitor cell contents in the post-thawing samples between the conventional and automated methods.

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Conventional method</th>
<th>Automated method</th>
<th>P</th>
</tr>
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<tbody>
<tr>
<td>TNC</td>
<td>97.3 ± 2.2</td>
<td>97.1 ± 1.7</td>
<td>0.649</td>
</tr>
<tr>
<td>7-AAD viability (%)</td>
<td>66.2 ± 8.0</td>
<td>68.4 ± 8.1</td>
<td>0.196</td>
</tr>
<tr>
<td>MNC</td>
<td>79.3 ± 6.6</td>
<td>81.9 ± 7.0</td>
<td>0.078</td>
</tr>
<tr>
<td>CD34+cell</td>
<td>91.0 ± 6.5</td>
<td>94.8 ± 3.4</td>
<td>0.001</td>
</tr>
<tr>
<td>CFU-GM</td>
<td>7.4 ± 5.8</td>
<td>12.3 ± 12.0</td>
<td>0.021</td>
</tr>
</tbody>
</table>

Conclusions: The automated BioArchive® system is superior to the conventional method, especially with regard to CD34+ cell viability and CFU-CM content.
"NORMAL" PLATELETS COUNT: IS IT RESULT REALLY CORRECT?
Gabriel Lima-Oliveira¹,², Francesco Dima², Martina Montagnana², Gian Luca Salvagno², Elia Citton², Gian Cesare Guidi¹,²
¹University of Parana Curitiba, Brazil, ²University of Verona Verona, Italy

Introduction: Falsely high platelet count have been reported as follows: i) after extreme burn injuries; ii) in bacteremia and malaria infection; iii) in conditions with pronounced microcytosis; iv) microangiopathic haemolytic anaemia, leukaemia, and lymphoma; and v) in cryoglobulinemia. Nevertheless, an “normal” platelet count (i.e., between 140–350 x 10⁹/L) is rarely linked as an laboratory error, thus may either misdiagnosis or delayed diagnosis of thrombocytopenia. This case report is aimed to describe a falsely normal platelet count in a thrombocytopenic inpatient. Methods: Case report: Results: In the morning of the patient admittance we performed a complete blood count (CBC) on the ADVIA2120i hematological analyzer, for a 79-year-old woman with a long history of thrombocytopenia (platelet count between 50 and 119 x 10⁹/L) HCV related. Surprisingly, platelets resulted in the normal range (383 x 10⁹/L). In the afternoon a new blood sample showed low count (87 x 10⁹/L). In the following morning a new platelet count was normal (282 x 10⁹/L) and in the afternoon platelet count resulted low (60 x 10⁹/L), both in K3EDTA and Na-citrate anticoagulated blood specimens. The third day complete blood analysis was performed at room temperature and after heating the sample for 30 minutes at 37°C. Blood counts were performed using two different blood cell counters: ADVIA2120i and Beckman Coulter UniCel® DxH 800. Both instruments reported falsely elevated platelet counts (282 and 337 x 10⁹/L, respectively) in specimens at room temperature. Contrarily, in specimens incubated at 37°C platelet count resulted low (87 and 84 x 10⁹/L, respectively). At room temperature the histogram of platelet volume distribution showed asymmetry in the platelet size histogram because the scatter of platelets detected cryoglobulins as small particles at the left of the diagram and both instruments highlighted with flags the presence of nucleated red blood cells. Blood film was prepared from specimen obtained at room temperature, stained according to the May-Grünwald-Giemsa technique and was examined at room temperature with a phase-contrast microscope. No precipitates of pale amorphous particles between red cells were macroscopically observed. Type II cryoglobulinemia HCV-related was confirmed by biochemical methods in serum sample. Accordingly, by immunofixation, the cryoglobulins precipitate contained monoclonal IgM, with κ light chains only, whereas IgGs components were polyclonal. Conclusions: In conclusion, since cryoglobulinemia is not a very rare phenomenon, especially in HCV patients, we recommend to identify and record affected patients and to perform CBC in specimens incubated at 37°C to guarantee patient safety.

MULTICENTER EVALUATION OF THE SYSMEX XN SERIES
Marianne Schoorl, Margreet Schoorl, Monique Chevallier, Tjeerd van der Ploeg, Hans van Pelt
MCrokenborgh, Holland

Introduction: The validation of hemocytometry equipment deviates significantly from those of clinical chemistry equipment due to the absence of appropriate control material and the need for fresh material. In practice, validation is limited to comparison with previously used equipment and determination of reproducibilities. Particularly in multicenter settings, uniformity is necessary. If the same equipment will be used in several laboratory departments, calibration and adjustment of the measuring channels is an important issue. In this study, 7 Sysmex XN Hematology modules distributed over three laboratories were evaluated with the same set of samples and simultaneously compared with the Sysmex XE-2100 Hematology analyzer whose performance has been extensively known from years of internal and external quality controls. Methods: Vacutainer® K₃EDTA anticoagulated samples from 160 patients were measured on all XN-modules and on the XE-module within four hours of collection. Results of WBC, RBC, PLT, Hb, Ht, MCV, MCH, MCHC, NEUT, LYMPH, MONO, EO, BASO, RDW and RETI of each Sysmex XN Hematology module were compared with the results of the Sysmex XE-2100 Hematology analyzer using linear regression. As criterion for approval was applied: 90% of the pairs of numbers must comply with the ideal line y = x with a 95% CI range dependent on the reproducibility of the parameter. Reproducibility of each parameter was determined in patient samples with low, normal and high levels. For statistical evaluation R, version 3.1.1 (R Foundation for Statistical Computing Platform) was used. Results: Reproducibility of all hematological parameters were all within the specifications of the manufacturer. The data sets of XE and XN results were plotted on the ideal line y = x with a 95% CI range, depending on the reproducibility of the concerning parameter. Subsequently, it was established whether the parameters of the 7 XN-modules corresponded to the XE-analyzer, or if there were differences. Also the average intercept, slope and correlation coefficient of the 7 modules were determined for each parameter. Only RBC and Ht from respectively 2 and 4 XN-modules had to be recalibrated. Conclusions: A straightforwardedmethod the WBC, RBC, PLT, Hb, Ht, MCV, MCH, MCHC, NEUT, LYMPH, MONO, EO, BASO, RDW and RETI results demonstrated on all 7 Sysmex XN Hematology modules uniform results, which were identical to the previously used Sysmex XE-2100 Hematology analyzer of which the performance was well known.
POSTER SESSION 2

100 - CELLULAR ANALYSIS
200 - COAGULATION
300 - FLOW CYTOMETRY
400 - MOLECULAR ANALYSIS
500 - MORPHOLOGY
600 - PLATELETS
700 - RED CELL ANALYSIS AND HEMOGLOBINOPATHIES
800 - STANDARDS AND QUALITY ASSURANCE

117 HOMOCYSTEINE LEVELS IN WOMEN WITH CERVICAL DYSPLASIA IN NIGERIA
Adewumi Adediran¹, Tolulope Ore², Vincent Osunkalu²
¹Department of Haematology, University of Lagos Lagos, Nigeria,
²Clinical Laboratory Department, Institute of Human Virology Nigeria. Lagos, Nigeria

Introduction: Homocysteine originates exclusively from the one-carbon-donating metabolism of methionine and it is remethylated into methionine with folates acting as the methyl donors. Homocysteine level is an indirect measure of folic acid, vitamin B12/ B6 status in man and hyperhomocysteinaemia reflects deficiencies of these vitamins. Impaired homocysteine metabolism negatively affects cellular growth, differentiation and function. This study was conducted to estimate serum homocysteine in women at risk of cervical carcinoma and to compare the mean plasma homocysteine level in this women with that of healthy women within the study population.

Methods: 150 overnight fasting blood samples were investigated for haematological parameters using the Auto-hematology Analyzer and their plasma estimated for homocysteine levels using Enzyme Immuno assay technique. These blood samples include 100 from women that have been cytologically proven with cervical dysplasia and 50 from women who were PAP Negative. The subjects attended the cervical screening centre of the Lagos University Teaching Hospital, Lagos.

Results: Higher levels of plasma homocysteine were associated with older females in the subject group (p=0.000). Elevated plasma homocysteine was not significantly associated with Hb, WBC, MCV, MCH, MCHC and RDW% in both the study group and control group. Elevated plasma homocysteine levels were significantly associated with High grade Squamous Intraepithelial Lesion compared (HSIL) where 62.5% of subjects had plasma homocysteine values higher than the mean values and there is no statistically significant difference in the mean value of homocysteine between the other classes of cervical epithelial changes (ASCUS & Low SIL) and the control group (mean=7.56, 7.94 and 8.38).

<table>
<thead>
<tr>
<th></th>
<th>MEAN HR (g/dL)</th>
<th>MEAN WBC (x10⁹/L)</th>
<th>MEAN RDW (%)</th>
<th>MEAN HOMOCYSTEINE (µmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONTROL GROUP</td>
<td>11.79</td>
<td>6.69</td>
<td>13.83</td>
<td>8.38</td>
</tr>
<tr>
<td>STUDY GROUP</td>
<td>11.85</td>
<td>6.51</td>
<td>13.8</td>
<td>8.32</td>
</tr>
<tr>
<td>P-VALUE</td>
<td>0.693</td>
<td>0.598</td>
<td>0.940</td>
<td>0.139</td>
</tr>
</tbody>
</table>

Conclusions: This reveals that hyperhomocysteinaemia may be associated with the development of cervical cancer due to increased proliferation of megaloblastic epithelial cells and quick utilization of folic acid and/or vitamin B12/B6. However, it is likely to be due to folic acid deficiency which leads to hyperhomocysteinaemia as the precancerous stage progresses to carcinoma in-situ.

118 EFFECT OF MALARIA-INDUCED HEME AND HEME OXYGENASE ON PREGNANCY OUTCOMES
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¹University Of Ghana Medical School Accra, Ghana, ²University of Ghana Medical School Accra, Ghana, ³Morehouse School of Medicine Atlanta, GA, USA, ⁴Morehouse School of Medicine Atlanta, GA, USA

Introduction: Plasmodium falciparum malaria threatens about 200 million people worldwide resulting in 655,000-1000000 deaths annually with pregnant women and children at high risk. Malaria in pregnancy causes severe maternal anemia, low birth weight deliveries and maternal and infant mortality. Although current anti-malarial treatment are effective in targeting parasites, recent studies have shown that the pathogenesis of severe malaria is not only due to parasitemia but also by parasite derived factors and host factors such as heme and heme oxygenase-1 (HO-1) as a result of hemolysis. Furthermore we have shown that heme and HO-1 are involved in the pathogenesis of experimental cerebral malaria. In this current study we determine the effect of malaria-induced heme and HO-1 on pregnancy outcomes. We hypothesized that pregnant women with placental malaria will have high levels of Heme/HO-1 and poor pregnancy outcomes than pregnant women without malaria.

Methods: We measured the Heme and HO-1 levels in plasma samples from pregnant women with and without malaria and correlate it with their pregnancy outcomes. Results: The preliminary results showed that pregnant women with malaria had significant higher mean levels of heme (80.160.4) than pregnant women without malaria (66.131.6), p = 0.006. Conclusions: Malaria in pregnancy is associated with increased Heme and HO-1 reflecting the degree of hemolysis induced by parasites (sequestered or systemic) and pregnancy outcomes. Findings from this study may provide insight in effect of malaria derived heme and HO-1 in pregnancy which may result in development of preventive chemotherapy that target both parasites and hemolysis or reduce the levels of heme in pregnancy during malaria infection.
EXPERT SYSTEMS IN HEMATOLOGY LABORATORY

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Introduction: Hematology was one of the first laboratory specialties where validation expert systems were applied, mostly oriented to sample selection for further investigation and/or microscopic morphological review. The validation rule is based on “operands” connected by computer operators and consisting in analyzers, microscopic workstations, LIS/middleware database advice. Methods: Constant application of a validation-rule set allows for each sample to set a decision path, highly available for analytical and clinical information. Three hematological cases are reported: 1) myeloproliferative disease; 2) interference in count; 3) hemoglobinopathy, to demonstrate as a practical example the facilities carried by dedicated middleware in the management of modern hematology laboratory. Results: -The operators' activity is “assisted and standardized” in all the involved health-network (hospital wards, laboratories,...), through a computing information grid managed by the LIS and dedicated to predefined case groups. It can promote a suitable management of a complex instrumental framework for everyone involved in common services (for example “on call”). -In first instance, some details are provided by archive data and interpretation rules related to previous instrumental results, test laboratory and clinical information from middleware repository. -Review rules and use of patient data must be studied properly in relation to the served characteristics of the population (i.e. age, race,...). -As to hematology laboratory, particularly interesting is the possibility, already present, of images storage. Conclusions: This facility shows three main aspects essential in management of hematologic diagnostic pathway: 1) a patient folder storing data and images can be done. This action sets the possibility to evaluate and to compare results, in follow up, also about morphological events/changes. Also comments, provided in previous reports, can be seen. This kind of facility will be available to all operators who need to manage the same patient in different access with a similar service approach, whatever the specific skills are. 2) Captured images can be sent to another reference/specialized center, etc.) for advice, engagement, morphologic and/or clinical counseling, second opinion consultants.

THE PREVALENCE OF CALR MUTATION IN MYELOPROLIFERATIVE NEOPLASMS AND THE COEXISTENCE WITH JAK2 MUTATION

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Introduction: Calreticulin gene (CALR) mutation has recently been demonstrated in myeloproliferative neoplasms (MPN) as a diagnostic marker. In initial reports, it was reported to be mutually exclusive with the JAK2 mutation, and most of the studies have involved only JAK2-mutation-negative patients. Here we studied the prevalence of CALR mutation in Korean patients with BCR-ABL negative MPN, the coexistence of JAK2 and CALR mutations and its clinical characteristics, and also the clinical characteristics of the two major types of CALR mutation. Methods: We first enrolled 126 BCR-ABL negative MPN patients. The cohort included 74 essential thrombocytopenia (ET), 4 primary myelofibrosis (PMF), 45 polycythemia vera (PV), and 3 chronic neutrophilic leukemia (CNL) patients. As we found the coexistence cases with JAK2 mutation in ET patients, we further enrolled 96 ET patients. JAK2V617F mutation was performed with real-time quantitative PCR, and JAK2 exon 12 and MPL exon 10 mutation were performed with direct sequencing. CALR mutation was performed first with fragment analysis for mutant allele burden calculation, and then with direct sequencing for confirming the mutation type. We compared the gender, age, laboratory results, splenomegaly, thrombotic events, plateletpheresis, and progression-free survival (PFS) divided in four groups: JAK2+/CALR+, JAK2+/CALR-, JAK2-/CALR+, and JAK2-/CALR. Results: The overall frequency of CALR mutation in BCR-ABL negative MPN was 19.8% with 31.1% in ET, 25.0% in PMF, 2.2% in PV, and 0% in CNL. In 167 ET patients, the coexistence of JAK2 and CALR mutations was detected in 7 (4%) patients. A total of eight types of CALR mutation were detected. The most common mutation types, type 1 (c.1092_1143del) and type 2 (c.1154_1155insTTGTC), were detected in 50% and 27% of ET patients, respectively. JAK2+/CALR+ patients showed no statistically different characteristics compared to other groups. Interestingly, JAK2+/CALR-patients showed older age (P= 0.008), higher leukocyte count (P< 0.0001), higher hemoglobin level (P= 0.0003), and more frequent thrombotic event (P= 0.004) compared with JAK2-/CALR-patients. There was no significant difference in PFS between the four groups. Patients with type 2 CALR mutation showed more thrombotic event than with type 1 mutation (P= 0.044). Conclusions: The prevalence of CALR mutation in BCR-ABL negative MPN seems to be lower than the prevalence reported in Western regions. JAK2 and CALR mutations can coexist in ET patients, but this double-mutant group showed no significantly different phenotype and clinical course, distinct from the other groups. In addition, the JAK2 mutation might have more impact on clinical and laboratory features than the CALR mutation. The patients with type 2 CALR mutation showed more thrombotic event than those with type 1 mutation.
121 EVALUATION OF THE ABNORMAL CELL FLAGS ON THE SYSMEX XN AND SYSMEX XE-5000 ANALYZERS IN HEMATOLOGICAL PATIENTS WITH BLASTS OR ABNORMAL LYMPHOCYTES IN PERIPHERAL BLOOD.
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Donostia University Hospital Donostia, Spain

Introduction: The new Sysmex XN analyzer has introduced new channels and reagents for better cells differential and abnormal cell flagging. WNR (white cell nucleated channel) counts NRBC and basophils and WPC (white precursor channel) separate blasts flag from abnormal lymphocytes flag. Sysmex XE-5000 include flags Blasts (B) and Abn Lympho/L_Blasts (AbL-B). Sysmex XN include flags Abn Lympho (AbL) and Blasts (B). Both analyzers can flag Atypical Lympho (AL). Methods: Between Nov 2014 and Jan 2015 we evaluated abnormal cell flags in the Sysmex XE-5000 and Sysmex XN analyzers in 104 patients with haematological disorders with presence of blasts (26 with AML, ALL or MDS), abnormal lymphocytes (31 with typical CLL, 26 with atypical CLL, and 17 with other lymphoproliferative disorders) or plasmatic cells (4 myeloma) in peripheral blood. Results: Results are presented in the following table.

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>No flags AbL-B</th>
<th>No flags B</th>
<th>AbL AL</th>
</tr>
</thead>
<tbody>
<tr>
<td>AM-L+ALL-MDS</td>
<td>26</td>
<td>6</td>
<td>7 13</td>
<td>0</td>
</tr>
<tr>
<td>TYPICAL CLL</td>
<td>31</td>
<td>2</td>
<td>0 29</td>
<td>1</td>
</tr>
<tr>
<td>ATYPICAL CLL</td>
<td>26</td>
<td>1</td>
<td>0 25</td>
<td>1</td>
</tr>
<tr>
<td>MYELOMA</td>
<td>4</td>
<td>0</td>
<td>0 1</td>
<td>4</td>
</tr>
<tr>
<td>OTHER LD</td>
<td>17</td>
<td>3</td>
<td>1 13</td>
<td>0</td>
</tr>
</tbody>
</table>

Left column: Flags from Sysmex XE-5000, Right column: Flags from Sysmex XN. Some samples have more than one flag. (B): Blasts, (AbL-B): Abn Lympho/L_Blasts, (AbL): Abn Lympho (AL): Atypical Lympho. Conclusions: In AML+LAL+MDS Sysmex XE-5000 flags more for AbL-B than for B; XN flags meanly for B. Both analyzers have problems to flag samples with 1-2 % blasts, and occasionally with more blasts. In typical CLL, Sysmex XE-5000 flags for AbL-B while Sysmex XN discriminates better for abnormal lymphocytes with AbL flagging. In atypical CLL, results are similar, but in some samples Sysmex XN flags for B +/- AL. In other LD, Sysmex XE-5000 flags for AbL-B, and the XN flags varied. In 2 hairy cell leukemias none of the analyzers flagged. In myelomas Sysmex XE-5000 flags for AL while Sysmex XN flags may vary. Abnormal lymphocytes flagged for false basophilia in three cases (two atypical CLL and one Sézary syndrome) in Sysmex XE-5000 whereas there were no cases in Sysmex XN.

122 CLINICOPATHOLOGICAL IMPLICATIONS OF MITOCHONDRIAL GENOME ALTERATIONS IN PEDIATRIC ACUTE MYELOID LEUKEMIA
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Introduction: The association between pediatric acute myeloid leukemia (AML) and mitochondrial aberrations has not been previously examined. In this study, we investigated various mitochondrial aberrations in pediatric AML, and evaluated their impacts on clinical outcomes. Methods: Sequencing, mtDNA copy number determinations, mtDNA 4 977-bp large deletion assessments, and gene scan analyses were performed on the bone marrow mononuclear cells of 55 pediatric AML patients and on the peripheral blood mononuclear cells of 55 normal controls. Changes in the mitochondrial mass, mitochondrial membrane potential, and intracellular reactive oxygen species (ROS) levels were also examined. Results: mtDNA copy numbers were about two-fold higher in pediatric AML cells than controls (P < 0.0001). Furthermore, a dose-response relationship was found between mtDNA copy number tertiies and the risk of pediatric AML, and intracellular ROS levels, mitochondrial mass, and mitochondrial membrane potentials were all elevated in pediatric AML. The frequency of the mtDNA 4 977-bp large deletion was significantly higher in pediatric AML cells, and pediatric AML patients harboring high amount of mtDNA 4 977-bp deletions showed shorter overall survival and event free survival rates, albeit without statistical significance. Conclusions: These findings demonstrate an association between mitochondrial genome alterations and the risk of pediatric AML.
123 USE OF DELTA NEUTROPHIL INDEX FOR DIFFERENTIATIONAL DIAGNOSIS OF LOW-GRADE COMMUNITY-ACQUIRED PNEUMONIA FROM UPPER RESPIRATORY INFECTION

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Introduction: This study aims to evaluate the diagnostic power of delta-neutrophil index (DNI) (figure 1) for the differentiation of low-grade community-acquired pneumonia (CAP) from upper respiratory infection (URI) in patients with clinically ambiguous symptoms. Methods: In brief, patients with CAP and URI were included in the Uijeongbu St. Mary’s Hospital, the Catholic University of Korea from September 2013 through November 2013. Inclusion criteria of low-grade CAP is the patients who showed the low severity in the CURB-65 system (score 0 or 1). URI is defined by acute infections involving the nose, paranasal sinuses, pharynx, larynx, and trachea, and the prototype is the illness without radiological pulmonary infiltrate. The patients with non-infection were used as the control group. Total and differential leukocyte counts, and the DNI values were obtained using an automated hematology analyzer, ADVIA 2120i (Siemens Healthcare Diagnostics, Germany). CRP was measured using a Hitachi 7600 Analyzer (Hitachi, Japan). Microbiologic tests were performed by sputum and blood specimens. Results: The numbers of individuals in the CAP, URI, and control groups were 59, 30, and 38, respectively. The number of zero score among the patients with CAP in accordance with CURB-65 system was 30 and the number of patients with 1 score was 29. The median (first – third interquartile range) of biomarkers of low-grade CAP, URI and control groups were shown in figure 1 (A CAP, B URI, C control groups). For the prediction of CAP, the CRP, lymphocyte and DNI showed the high area under the curves (AUCs) among the tested biomarkers (AUC 0.845 for DNI, 0.926 for CRP, 0.557 for WBC, 0.780 for neutrophil, 0.896 for lymphocytes, and 0.704 for monocytes). The optimal cutoffs for the prediction of CAP were >1.7% for DNI, >1.48 mg/L for CRP, >5.23x10$^{3}$/µL for neutrophil and ≤ 1.57x10$^{3}$/µL for lymphocytes. The combination of DNI, CRP and lymphocytes showed higher AUC (AUC 0.974, sensitivity 91.5%, specificity 95.6% for the prediction of CAP). Conclusions: DNI showed significantly higher results in lower-grade CAP group than in the URI and control groups. Total leukocytes and neutrophils showed less diagnostic power for differentiating CAP from URI than DNI. Furthermore, the combination of DNI, CRP, and lymphocytes showed the highest diagnostic power than each of the biomarkers independently. Therefore, DNI may be helpful to do appropriate treatment in patients in need of antibiotics and prevent unnecessary abuse of antibiotics in patients with ambiguous clinical symptoms.

124 PROPOSAL FOR RECOMMENDATIONS FOR THE USE OF THE MEAN PLATELET SIZE(MPS) IN FLOW CYTOMETRY (FCM) TO STANDARDIZE THE MEAN PLATELET VOLUME(MPV) IN AUTOMATED HEMATOLOGY ANALYZERS(HAS)

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Blood Cell Count Standardization Subcommittee, Japanese Society for Laboratory Hematology Tokyo, Japan

Introduction: Increased MPV obtained by HA is associated with increased risk of myocardial infarction independent of known cardiovascular risk factors. It became to raise expectations for a useful marker. However the standardization was difficult. One reason why MPV analysis is not widely used was that the values were unstable after blood sampling. Another reason was that the values of MPV depended on each different detecting principle and the reagent of HAs. Therefore the definitions of MPV axis on the platelet size histogram were not completely same in each manufacturer. The MPV standardization may lead to it being a useful marker in clinical practice in the future. The following standardization was needed. 1) Standardize the condition for pre-analytical procedure. 2) Define the lower limit threshold for deriving MPS. 3) Establish the slope by regression analysis after converting MPS by dimension factor(DF). 4) Corresponding reference intervals to MPS by normalizing with mean of HAs’ MPVs. Methods: For determination of MPS, we improved the ICSH-ISLH documents for platelet counting(2001), and it was validated. The new SOP was used for determination of MPS. We analyzed these data as the following. 1) Establish procedure to derive MPS including DF and Slope. Establish standardization by regression analysis and calculation of converting axis by DF(n=53, HA=1, FCM=1). 2) Validation the MPS and the procedure(n=3, HA=6, FCM=7). Antibody to identify for platelets was used CD61 and CD41. Paraformaldehyde(1%) was used for reducing the influence of natural activation of the platelet. Flowcytometric analysis was performed using seven FACS-Can-toI analyzers(BD Biosciences). Six manufacturers’ reference HAs in Japan were used. Determination of DFs and Slopes was used regression analysis between MPS$^c$ and the mean of MPVs as the intercept to zero, to determine DF and Slope. Results: Precision and %bias of MPS were less than approximately 2% among seven FCM analyzers. MPSs were normalized by mean of MPV of six HAs. The conversion factors(DFs,Slope) were 0.239-0.569 and 0.862-1.270, respectively. The correlation coefficients and intercepts were 0.95-1.00 and -0.03+0.01(IL). Conclusions: MPSs by FCM analyzers were normalized by mean of MPV(6 HAs). The meanings of DF and Slope which were depended on measurement system would be the difference of axis and the shape factor of each analyzer, respectively.

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IJLH © 2015 John Wiley & Sons Ltd, Int. Jnl. Lab. Hem. 2015 37 (Suppl. 2) 1-130
Introduction: During last decade 3 sub-populations of monocytes have been described in the human blood: classical, intermediate and non-classical monocytes, which can be differentiated with flow cytometry based on the expression of CD14 and CD16. The involvement of monocyte subpopulations in different pathological processes also was described, including sepsis, HIV infection, tuberculosis and cardiovascular disorders. Very limited information available about monocyte sub-populations in Chronic Lymphocytic Leukemia (CLL), but they seem to be important for tumor progression and immune suppression in CLL. The goal of our study was to compare CD16 negative and CD16-positive monocytes in CLL patients and in normal individuals. Also we aimed to compare the groups of CLL patients with bad and good prognosis. Methods: 58 patients with confirmed diagnosis of CLL were included in the study at the time of diagnosis. The comparison group included 307 donors. EDTA-anticoagulated blood sample were analyzed with CytoDiff™ panel according to the recommendation of the supplier (Beckman Coulter). The CytoDiff™ panel is a 5-color / 6-marker reagent that provides an extended 18-part white blood cell differential from whole blood specimens by flow cytometry. Among other WBC sub-populations, CytoDiff™ is able to provide the count of CD16-positive and CD16-negative monocytes in the peripheral blood. The expression of CD38 on B-lymphocytes was used as prognostic marker for CLL patients. Results: The absolute number of classical monocytes was similar in donors and in CLL patients (503 cells/mcl vs 510 cells/mcl respectively). When compared to normal individuals, patients with CLL were characterized by higher absolute count of CD16-positive monocytes (40 cells/mcl vs 30 cells/mcl, p=0.0007), higher proportion of CD16-positive monocytes among all monocytes (8.8% vs 5.8%, p< 0.0001) and lower proportion of CD16-negative monocytes among all monocytes (91.3% vs 94.2%, p< 0.0001). Within the group of 58 CLL patients we identified patients with unfavorable prognosis (n=16) based on the CD38 expression (>6%). In patients with bad prognosis CytoDiff™ results revealed higher proportional count (0.24% vs 0.175%, p=0.1346) and absolute count (63 cells/mcl vs 38 cells/mcl, p=0.166) of CD16-positive monocytes, but the difference did not reach the statistical significance. Conclusions: CytoDiff™ analysis is very efficient tool to monitor the monocyte subpopulations in various pathologies, including CLL. The exact role of CD16-positive monocytes in CLL and their prognostic significance remain to be investigated on a larger cohort of patients. *Not available in the United States and other geographies.

Introduction: The Unicel DxH 800 Coulter Cellular Analyzer with VCSn(volume,conductivity,and light scatter)technology can provide morphologic properties of peripheral leukocytes,known as cell population data(CPD).The aim of this study was to evaluate the usefulness of peripheral lymphocyte CPD for the differential diagnosis of viral infection from normal control,acute bacterial infection,and tuberculosis in adult person. Methods: Peripheral blood was collected from 90 patients with confirmed diagnoses of viral infections(Epstein-Barr virus,51 cytomegalovirus 22 hepatitis E virus,7 Parovirus ,2 other viruses ),70 patients with acute bacterial infection,22 tuberculosis patients and 100 normal controls.The lymphocyte CPD include the mean (MN) and standard deviation(SD) of the volume(V),conductivity(C),five light –scatter measurements . 14calculated CPD parameter values were obtained using a Unicel DxH 800 Coulter Cellular Analyzer. Results: The mean volume standard deviation of lymphocyte (LV-SD) were higher in the viral infection than the acute bacterial infection and tuberculosis(p< 0.01 for both),ROC curves evidenced excellent sensitivity in LV-SD with a cut-off of 18.03 (sensitivity of 87.5% ,specificity of 79.2% ) in diagnosing viral infection,while other parameters including mean conductivity,upperlowermedian angle light scatter,mean axial light loss and standard deviation achieved 71%-76% sensitivity and 55%-65% specificity in ROC. Conclusions: Since it could be readily obtained from hematology analyzer during routine automatic leukocyte differentials, the CPD parameters of lymphocyte could be useful in the detection of viral infection.In this study, the LV-SD may help clinicians in the diagnosis of virus infection.
Introduction: Examination of peripheral blood smears (PBS) is often an essential component of laboratory investigation of disease, but can be time-intensive and subjective, even for experienced morphologists. The CellVision DM96 (Lund, SE) is an automated slide scanner which uses image analysis to pre-classify WBCs. However RBC morphology is not fully assessed limiting full potential for maximizing efficiency since manual light microscopy (MLM) review is still required. A new Advanced RBC Application (Advanced RBC) expands the DM96’s utility by using a neural network to pre-classify RBC by size, shape, color, and inclusions. In this study we compare RBC morphology results from Advanced RBC to MLM. Methods: 233 Wright stained PBS were chosen to include a variety of abnormalities of RBC size, shape, color, and inclusions. Each was examined by 1 of 3 experienced technologists using the DM96 with Advanced RBC. Technologists were able to reclassify RBCs they felt were misclassified. Results were compared to the same technologist’s MLM results or RDW (Sysmex XE-5000) for anisocytosis. Sensitivity and specificity were calculated for detection of anisocytosis (RDW ≥15%), hypochromic RBCs (≥7%), polychromatic RBCs (≥2%), dacrocytes (≥1%), drepanocytes (≥1%), codocytes (≥7%), schistocytes (≥1%), ovalocytes (≥18%), spherocytes (≥1%), acanthocytes (≥1%), Howell-Jolly bodies (≥1%), Pappenheimer bodies (≥1%), basophilic stippling (≥2%), and parasites (≥1%). Weighted K statistics were also calculated. Results: Table 1 summarizes RBC morphology results using the Advanced RBC compared to MLM. Highest agreement between Advanced RBC and reference method was seen for shapes and anisocytosis. Sensitivities for anisocytosis and shapes were also highest, including 90.8% sensitivity for schistocytes. Ks were high for abnormal shapes, especially codocytes (0.7) and drepanocytes (0.8).

<table>
<thead>
<tr>
<th>Abnormality</th>
<th>Raw agreement %</th>
<th>Specificity %</th>
<th>Sensitivity %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anisocytosis</td>
<td>92.6</td>
<td>84.2</td>
<td>95.4</td>
</tr>
<tr>
<td>Color</td>
<td>86.3</td>
<td>87.9</td>
<td>85.2</td>
</tr>
<tr>
<td>Shape</td>
<td>90.1</td>
<td>90.4</td>
<td>89.8</td>
</tr>
<tr>
<td>Inclusions</td>
<td>88.4</td>
<td>90.2</td>
<td>84.1</td>
</tr>
</tbody>
</table>

Conclusions: Interpretation of RBC morphology using the DM96 with Advanced RBC is comparable to interpretation by MLM. Sensitivity for abnormal color and inclusions is slightly lower than for size and shape, possibly due to resolution of images and/or computer displays. Implementation of this software, particularly with full automation, has potential to greatly improve workflow efficiency and standardize reporting in hematology laboratories.
129 CLINICAL VALIDATION OF AUTOMATED CSF CELL COUNTS ON THE SYSMEX XN HEMATOLOGY ANALYZER
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2Sysmex America, Inc. Lincolnshire, IL, USA

Introduction: Recent implementation of Sysmex XN analyzers presented the opportunity for comparison between manual, Sysmex XE-5000 (XE), and XN methods to determine if XN was superior to XE for CSF cell counts. A secondary objective was to assess the clinical impact of reporting automated CSF cell counts from the XN analyzer. Methods: Forty-three (43) samples were analyzed by all 3 methods and 22 additional samples (total = 65) were analyzed by manual and XN methods after the XEs were removed. XE and XN analyses were performed immediately after manual cell counts. Cytospins and manual differential counts were performed on all samples. Deming regression and truth table analyses were done using manual cell counts as the reference method. Results: There were 65 samples from 44 patients; 11 patients had 2 samples. 55 samples were described as clear and 10 as hazy or cloudy. For WBC counts, XN and XE analyzers showed excellent correlation with each other (r = 0.98). The XN results showed a stronger correlation with manual WBC counts than the XE (r = 0.92 vs. r = 0.89). The sensitivity of both analyzers for detection of WBC > 5/μL was 100%. However, the XN showed a specificity of 86% in comparison to 58% for the XE. 14 samples from 11 patients with acute leukemia had manual WBC ranging from 0-1/μL and XN WBC ranging from 0-17/μL. Cytospins smears on all 14 samples were negative for leukemic blasts or meningitis. Ten samples were from 10 patients with clinical suspicion of meningitis; 3 had significantly elevated WBC (75-450/μL) by manual and XN methods, and 7 were negative by both methods. Correlation of WBC differential counts was limited due to low cell counts in the majority of samples. Comparison of RBC counts was also limited, because the XN produces RBC counts in increments of 1,000/μL; however, the 55 clear CSF samples had RBC ≤ 1,000/μL by manual and XN methods, and 8 of 10 cloudy or hazy fluids had XN RBC ≥ 2,000/μL. Six of these 8 samples were from patients who had recent brain surgery or intra-cranial hemorrhage and 2 were consistent with peripheral blood contamination. Conclusions: CSF WBC counts performed on XN show improved specificity for true negative samples compared to the XE. No samples from patients with acute leukemia, suspicion of meningitis, or intra-cranial hemorrhage would have been mis-classified using XN cell counts. Clear CSF samples have XN RBC counts ≤ 1,000/μL.

130 SENSITIVITY OF THE SYSMEX XN9000 WPC-CHANNEL FOR DETECTION OF MONOCLONAL B-CELL POPULATIONS
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MCA Alkmaar, Netherlands

Introduction: Hematology analyzers generate suspect flags if abnormal cells are present in the samples. With the introduction of the Sysmex XN Haematology analyser the white blood cell differential channels (WNR and WDF) and abnormal cell detection channel (WPC) have been introduced with improved algorithms for flagging blasts and abnormal lymphocytes. In this study the performance characteristics of the WBC suspect flags were compared with immunophenotyping results. Methods: Blood samples from 55 subjects (age > 30 year) with lymphocytosis (≥ 4.2 x 10^9/L) were evaluated. In 45 cases an additional WPC reflex test was performed automatically. The Sysmex XN analyser was equipped with software version 00.15. Q-flag settings were according to the specifications of the manufacturer. Immunophenotyping was performed on all samples with a Beckman Coulter FC500 flowcytometer. According to the WHO criteria (2008) for immunophenotyping, cases were classified in subgroups with B-ALL, B-CLL, B-NHL, B-NHL/Hairy Cell Variant, MBL and AML. In case of MBL only a small monoclonal B-cell population (< 5.0 x 10^9/L) is present. Results: 1. WPC positive flagging. After the WPC reflex test a positive Blasts flag was demonstrated in 8 cases, resulting in 5xAML, 1xB-ALL, 1xB-CLL and 1xB-NHL. The Abnormal Lymph flag was demonstrated in 23 cases, resulting in 13xB-CLL, 1xB-NHL, 1xB-NHL/Hairy Cell Variant, 6xMBL and 2x cases without a monoclonal B-cell population. Only one case demonstrated an Atypical Blasts flag. 2. WPC negative flagging. After the WPC reflex test a negative flag was demonstrated in 13 cases. Immunophenotyping resulted in 1xB-CLL, 3xB-NHL, 8xMBL and 1 case without monoclonal B-cell population. 3. Lymphocytosis without WBC flagging. In 10 cases with lymphocytosis no flag was demonstrated. Immunophenotyping resulted in 4 cases with MBL and 6 cases without monoclonal B-cell population. In 16 of the 23 cases without WBC flagging a monoclonal B-cell population was detected. In only 6 of the 18 MBL cases the WPC channel demonstrated a positive flag: Abnormal lymph. Conclusions: Advanced algorithms combining lymphocytosis with suspect flagging is needed in order to improve the sensitivity of the WPC channel for detection of monoclonal B-cell populations.
131 IMPACT OF INTEGRATING RUMKE STATISTICS TO ASSIST WITH CHOOSING BETWEEN AUTOMATED HEMATOLOGY ANALYZER DIFFERENTIALS VERSUS MANUAL DIFFERENTIALS
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Introduction: Judicial limitation of manual differentials increases hematology laboratory efficiency and reduces discrepancies between automated absolute neutrophil counts released with the blood counts before posting of the complete differential, an increasingly common practice in many laboratories serving cancer center infusions. Our institutional hematology laboratory procedure states the clinical laboratory scientist should post the automated differential rather than the manual differential if the former is within the 95% confidence interval of the latter, as determined by the “Rumke Statistic” (Rumke, CL. College of American Pathologists CAP Conference, Aspen, CO, 1977; 39-45). However, applying this in real-time laboratory practice is impractical and time-consuming. Retrospectively applying the Rumke statistics to the hematology laboratory posting algorithm, we analyzed the potential impact of real-time use of Rumke statistics for more judicious use of the automated versus manual differential and for reducing discrepancies between the instrument absolute neutrophils count (IANC) and manual absolute neutrophil count (ANC).

Methods: Complete blood counts with automated differentials produced by a Sysmex XE5000 for the Moores UCSD Cancer Center over a one-week period were compared with their 100-cell manual differentials using the Rumke 95% confidence intervals as calculated using the Clopper-Pearson method. All calculations were performed using the open source statistical programming language R (www.r-project.org).

Results: Of 1121 complete blood counts with differentials posted, 155 had manual differentials performed. According to the Rumke analysis, 57 (36.8%) of these posted manual differentials had 95% confidence intervals that included the non-posted autodifferentials. Fifty-nine (38.1%) of the manual differentials had discrepancies between the IANC and ANC, potentially resulting in infusion center errors. Among all samples, 42 had ANCs below 1500/uL, a threshold for most infusion decisions that are dependent on neutrophil counts. Of these, 12 (28.6%) had IANC-ANC discrepancies, all of which were underestimated by the IANC. These discrepant cases also had instrument overestimations of lymphocytes (100%), monocytes (75%), and immature granulocytes (41.7%). Hematopathologist (HEB) review of the blood smears for these IANC-ANC discrepant cases revealed that only one of the 12 truly needed a manual differential.

Conclusions: A real-time operator alert concerning the statistical identity of manual versus automated differentials could have reduced manual differentials by nearly 37%. Not posting the manual differential for the cases with low ANCs and IANC-ANC discrepancies would have likely reduced clinical error/confusion.

132 HYPOCHROMIC CELLS BY SYSMEX XN IN THE DETECTION OF FUNCTIONAL IRON DEFICIENCY
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1HOSPITAL GALDAKAO USANSOLO GALDAKAO VIZCAYA, Spain, 2Hospital Universitario Basurto Bilbao, Spain

Introduction: Consequence of the imbalance between the iron requirements of erythroid and the actual supply is a reduction of red cell hemoglobin content, which causes hypochromic cells reticulocytes and after maturation erythrocytes. Sysmex XN analyzer (Sysmex Corporation, Kobe, Japan) reports reticulocyte hemoglobin equivalent (Ret He) and the percentages of erythrocyte subsets, including the hypochromic fraction (%Hypo He). We aimed to study the value of these parameters of hemoglobinization in the detection of functional iron deficiency. Methods: Seventy healthy subjects, 40 patients on hemodialysis (HD) and 35 peritoneal dialysis (PD) receiving therapy and 80 patients with iron deficiency anemia (IDA) were analysed. Samples were obtained in the course of routine treatment and good iron availability, MCH within reference range, %Hypo He) and iron restricted erythropoiesis (low Ret He). HD and PD patients showed efficient erythropoiesis, maintained due to treatment and good iron availability, MCH within reference range, Ret He > 30 pg and %Hypo He slightly increased. The results of ROC curves analysis for the diagnosis of iron deficiency were Ret-He AUC 0.84 cut off 30.8 pg, sensitivity 78.7 %, specificity 87.2 %, % Hypo He AUC 0.78 cut off 2.5 %, sensitivity 72.2 %, specificity 88.1 %. Conclusions: %Hypo He and Ret He provide information about individual cell characteristics, the hypochromic cells are detected and quantified improving the evaluation of erythropoiesis and iron status.
THE EXPRESSION OF SLAMF7 LEVELS IN MALIGNANT B CELLS: A NOVEL THERAPEUTIC PATHWAY FOR PATIENTS WITH CLL

Introduction: Activation of SLAMF7 leads to proliferation or differentiation of the B-cells; a receptor found on the surface of B-lymphocytes. It is hypothesised that SLAMF7, mostly found in multiple myeloma cells, can also be found and upregulated in B chronic lymphocytic leukaemia cells (B-CLL). Here we look at the possibility of upregulating the level SLAMF7 expression on the B-CLL cells, with phorbol myristate acetate (PMA) and Bryostatin and to determine the optimal dose for upregulation using Bryostatin. Methods: B-CLL cells were cultured with RPMI 1640 to increase the number of SLAMF7 receptors on the cell surface and the white cells were extracted with histopaque. The levels of expression of SLAMF7 cells were measured, using immunofluorescence, flow cytometry, confocal microscopy and reverse transcriptase polymerase chain reaction (RT-PCR). The effects of treatments with PMA and Bryostatin were measured. Further test was done to determine the optimal dose response for upregulation of SLAMF7 receptor using Bryostatin. Results: Varying levels of SLAMF7 expression were identified with different B-CLL cells. Some cases showed measurements of high expression levels while untreated. There was higher expression levels associated with PMA; which was further demonstrated by showing more fluorescence with confocal microscopy. Lesser expression levels were associated with Bryostatin relatively. The dose response test further demonstrated that 10nM of Bryostatin gives the optimal upregulation of the SLAMF7 receptors.

Table 1: Bryostatin Dose Response Values using flow cytometry for selected cases in the study

<table>
<thead>
<tr>
<th>Conc.</th>
<th>0 nM</th>
<th>10 nM</th>
<th>20 nM</th>
<th>40 nM</th>
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</thead>
<tbody>
<tr>
<td>%Pos</td>
<td>0.94</td>
<td>1.32*</td>
<td>0.78</td>
<td>0.61</td>
</tr>
<tr>
<td>MFI</td>
<td>5.52</td>
<td>5.54*</td>
<td>5.19</td>
<td>5.09</td>
</tr>
<tr>
<td>%Pos</td>
<td>6.94</td>
<td>16.11*</td>
<td>15.91</td>
<td>4.02</td>
</tr>
<tr>
<td>MFI</td>
<td>3.56</td>
<td>5.31*</td>
<td>5.29</td>
<td>4.02</td>
</tr>
<tr>
<td>%Pos</td>
<td>13.37</td>
<td>12.42*</td>
<td>11.81</td>
<td>5.2</td>
</tr>
<tr>
<td>MFI</td>
<td>4.47</td>
<td>4.04*</td>
<td>3.62</td>
<td>1.87</td>
</tr>
</tbody>
</table>

Conclusions: Both PMA and Bryostatin could upregulate SLAMF7 receptors on B-CLL cells hence may be a possible therapeutic pathway for treatment of CLL patients.

SAME-DAY DUPLICATE ORDERS FOR COMPLETE BLOOD COUNT WITH DIFFERENTIAL
Megan Nakashima, Yang Shi, Eric Hsi
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Introduction: The complete blood count with leukocyte differential (CBCDIF) is a commonly ordered test. Although automated analyzers usually provide accurate and efficient results, some samples require manual blood smear review at significant increase in cost and time. We examined duplicate CBCDIF orders (orders placed < 24 hours after a previous CBCDIF on the same patient) to investigate what impact these had on laboratory workflow and assess patterns of ordering which may indicate target areas for improvement. Methods: We queried our laboratory information system (Sunquest, Tucson AZ) for duplicate CBCDIFs on inpatients during one month. Data was collected including ordering physician, location, if the previous result was available at the time of the second order, and differences in results between initial and duplicate tests. Results: 1367 duplicate CBCDIF were ordered on inpatients in May 2014, accounting for 9% of inpatient CBCDIF orders. 355 had manual differentials performed per laboratory policy requiring an estimated 60 hours of technologist time.The duplicate was usually ordered by the same physician (77.4%) and from the same unit (90.9%) as the previous order. In 66.5% of cases, previous results were finalized when the duplicate was ordered. Duplicate CBCDIF orders accounted for >15% of all CBCDIF orders in 10 hospital units (9 intensive care units [ICUs] and the colorectal surgery unit). Changes between results from the first and duplicated test ordered from these units (n=735) are summarized in the table. 440 patients had a change of absolute neutrophil count (ANC) of ≥1.0 x106/L; however this was reflected in a ≥1.0 x106/L change in white blood cell count (WBC) in 89% of cases.

<table>
<thead>
<tr>
<th>Absolute change between duplicates</th>
<th>Hemoglobin (g/dL)</th>
<th>Platelets (x10^9/L)</th>
<th>WBC (x10^9/L)</th>
<th>ANC (x10^9/L)</th>
<th>ALC (x10^9/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (STDEV)</td>
<td>0.723 (0.801)</td>
<td>27.298 (33.066)</td>
<td>2.181 (2.370)</td>
<td>2.027 (2.126)</td>
<td>0.428 (0.497)</td>
</tr>
</tbody>
</table>

Conclusions: Duplicate CBCDIFs constitute a significant portion of inpatient CBCDIFs at our institution and require at least 2 hours of technologist time/day to process. Duplicates are usually not a result of transfer of care between physicians or units, or because previous results are unavailable. Although absolute values sometimes varied widely between the first and duplicated CBCDIF, most of these changes, including 89% of changes in ANC, can be detected by CBC alone. Interventions to decrease duplicates should be targeted at ICUs and could include electronic medical record alerts displaying previous results and education about the utility of CBC without differential.
210 ANALYSIS OF THROMBIN GENERATION DATA: EFFECTS OF CALIBRATION AND ALPHA-2-MACROGLOBULIN CORRECTIONS
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Introduction: Thrombin generation (TG) is pursued as a global hemostasis assay. Debate remains regarding how to use TG to best represent the samples’ physiological behavior. Commercial TG analysis software implements correction for the alpha-2-macroglobulin (a2MG)-thrombin signal and an internal thrombin calibrator approach to correct for the inner filter effect (IFE), substrate consumption, and other fluorescence artifacts. This work analyzes TG data from samples reflecting a broad range of coagulation potentials to assess the impact of these corrections on thrombin generation parameters. Methods: TG assays were performed on normal pooled plasma using the Calibrated Automated Thrombinography platform (Stago, USA) as described in Machlus KR et al. Thromb Haemost 2009;102(5):936. Commercial and in-house software analyses were used to selectively compare contributions of various correction algorithms as described in Woodle SA et al. Thromb Res 2013;132(3):374. Results: Thrombin peak heights stayed near the linear portion of the thrombin calibrator’s fluorescence response. Time parameters and thrombin peak height returned essentially the same number regardless of what if any algorithms were used for calibration and a2MG-thrombin signal correction. The thrombin calibrator algorithm was able to correct for some of the run-to-run variations. In all TG parameters, inconsistent thrombin calibration was observed in the most procoagulant samples (highest thrombin peak) where substrate consumption occurred before the end of the experiment. Conclusions: With the exception of extremely procoagulant samples, substrate consumption and IFE did not significantly influence thrombin generation parameters. Thrombin peaks remained largely within the linear regime of the calibrator curves, but in samples exhibiting substrate consumption and IFE, calibration “corrected” the curves such that peaks fell near or into the range where the calibrator could not measure, rendering that output erratic or mathematically implausible. a2MG-thrombin correction does not affect TG parameters except possibly ETP; further studies are needed. This finding of the minimal impact of TG correction algorithms is consistent with our results using different deficient plasmas, but requires confirmation in multi-laboratory studies using clinically relevant samples. Disclaimer: This is an informal communication and represents authors’ best judgment. These comments do not bind or obligate FDA.

211 ALTERED HEMORHEOLOGICAL INDICES IN A NIGERIAN GERIATRICS POPULATION
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Introduction: Aging is a normal process of adult life characterised by a gradual decline in the physiologic reserves. Physiologic functioning is probably a better measure of aging than chronological age. Elevated plasma fibrinogen concentration has been linked severally with cardiovascular diseases and stroke in the elderly and the influence of plasma Fibrinogen concentration on plasma viscosities is well understood. Our interest was primarily based at establishing Fibrinogen index in Geriatrics especially in the Nigeria and secondly to assess their rheological parameters as probable indices of their cardiovascular function. Methods: A total of 50 apparently healthy, elderly Nigerians with ages between 60 and 85 years comprising of 25 males and 25 females recruited from old people’s home and some individuals around the southern area of Nigeria were studied for hemorheological parameters such as: Packed cell volume (PCV), Plasma and whole blood viscosity (PV and WBV respectively), Erythrocytes sedimentation rate (ESR), Plasma Fibrinogen concentration (PFC) and Euglobulin lysis time (ELT). They were compared with 50 healthy younger sex-matched subjects (controls). Results: We observed statistically significant increases in the values of PV, PFC, ESR and ELT while PCV and WBV exhibited significant decreases when compared with controls (P< 0.05, respectively). The increase in ELT is interpreted as a reduction in fibrinolytic activity. Conclusions: Decreased haematocrit and lowered whole blood Viscosity coupled with hyperfibrinogenaemia with subsequent high plasma viscosity and hypofibrinolysis could be reflections of advanced age. The decreased parameters may favour improved hemorheology at the onset but the overwhelming influence of the elevated parameters are indicative of abnormal rheology and a predisposition to thrombotic tendencies and other cardiovascular complications at old age. The need for exercise and prophylactic antithrombotic therapy especially at advanced ages may be indicated.
212 PERCEIVED CLINICAL UTILITY OF ADAMTS-13 TESTING DECREASES QUICKLY WHEN TURNAROUND TIME EXTENDS BEYOND TWO DAYS.
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Introduction: ADAMTS-13 Activity/Inhibitor testing is an esoteric coagulation test which is rarely done within hospital labs. The test is offered from a number of reference laboratories with turnaround times ranging from 24 hours to seven days. Because treatment decisions are often based on this test and patients with thrombotic microangiopathies are often in critical condition, it is worthwhile to examine physician attitudes toward ADAMTS-13 testing and turnaround times. Methods: An anonymous survey was sent via email to nephrologists and hematologists. Approximately 2,500 emails were sent with a link to the survey. The response rate for the survey was ~3% with 75 physicians responding. No compensation was offered. There were between 3 and 6 questions on each survey. Results: When asked “When evaluating a patient with a thrombotic microangiopathy, how critical do you find ADAMTS-13 testing in helping you to reach a diagnosis?” the responses showed this test is seen as very integral to reaching a diagnosis. “This test is critical” was selected by 38% of responding physicians, while 34% selected “This is an important component.” Only 5% of respondents selected “This test is only marginally useful.” No physicians selected “This test does not have any utility.” A question asking about the clinical utility with different turnaround times for ADAMTS-13 testing was phrased “Give your opinion on the clinical utility of receiving ADAMTS-13 results within the given time-frames.” Turnaround time options were from one day post-draw to seven days post-draw. Faster turnaround times were seen as more clinically useful with 91% of respondents saying a one day turnaround time was “Very Useful.” This number dropped to 58% finding a two day turnaround time “Very Useful.” A three day turnaround time was seen as “Very Useful” by only 14% of respondents. A five day turnaround time was seen as “Not Very Useful” or “Not Useful” by the majority of respondents with 37% selecting “Not Very Useful” and 25% finding the test “Not Useful.” Conclusions: ADAMTS-13 testing is seen as a very important laboratory test for nephrologists and hematologists differentiating thrombotic microangiopathies and the clinical utility of ADAMTS-13 testing is greatest when turnaround times are two days or less and clinical utility of the test rapidly diminished when turnaround times extend beyond two days.

213 CHALLENGES FACED WITH POINT-OF-CARE TESTING COAGULATION DEVICES - AN FDA PERSPECTIVE
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FDA Silver Spring, MD, USA

Introduction: Point-of-care testing (POCT) is commonly defined as diagnostic testing performed at or near the site of patient care. The growing use of POCT devices are designed to improve the access to medical testing and reduce test turnaround time. However, these advantages are accompanied by a number of challenges, and the growing use of POCT raises new challenges such as increased pre-analytic variability of POCT specimen types, sample matrix and stability considerations, operator training and testing in a less controlled testing environments. These variables may lead to deterioration of the quality testing results. Methods: This presentation will discuss the POCT spectrum, benefits and challenges of POCT and provide general considerations for device validation, along with recommendations for use of Clinical and Laboratory Standards Institute (CLSI) documents. Results: We list the challenges of POCT along with the resulting general considerations for analytical and clinical performance validation testing. Conclusions: We provide an overview of the FDA considerations for regulatory clearance of POCT coagulation testing devices to ensure balance between the perceived and/or realized advantages and disadvantages of POCT.
THE SEROTONIN RELEASE ASSAY SHOULD BE THE FIRST-LINE TEST FOR HEPARIN-INDUCED THROMBOCYTOPENIA IN PATIENTS WITH ANTIPHOSPHOLIPID ANTIBODIES

Nahla Heikal1,2, Crist Ronda2, George Rodgers1,2, Kristi Smock1,2
1University of Utah, Department of pathology SLC, UT, USA, 2ARUP laboratories SLC, UT, USA

Introduction: Antiphospholipid antibodies (aPL) have the capability to cause false-positive or indeterminate results in HIT immunoassays. However, whether aPL demonstrate platelet-activating properties in the functional serotonin release assay (SRA) has not been widely studied. The SRA is considered the gold standard test for the diagnosis of heparin-induced thrombocytopenia (HIT), with positive results reported to have >90% sensitivity and specificity for HIT. Indeterminate SRA results occur in approximately 2% of samples at our institution and usually represent non-heparin-dependent platelet activation. Indeterminate results are problematic because HIT-mediated platelet activation cannot be definitively identified or ruled out in these samples. We investigated whether samples containing high-titer aPL would cause false-positive or indeterminate reactivity in the SRA. Methods: 18 samples containing high-titer aPL antibodies (IgG, IgM, or IgA alone or in combination) against cardiolipin and/or B2 glycoprotein-I were tested in the SRA and with a polyclonal HIT immunoassay, including a high-heparin confirmatory step to evaluate heparin-dependent reactivity. Results: 6 samples (2 IgG, 2 IgM, 1 IgA, and 1 IgM and IgA aPL), showed positive results in the HIT immunoassay (OD values ranged from 0.429 – 1.887 with a cutoff for positivity of 0.399). 2 of these samples (1 IgM, and 1 IgA aPL) were considered heparin-dependent by the confirmation step (75% and 89% inhibition, respectively, with a cutoff of 50% for heparin-dependence). All 18 samples containing high-titer aPL were SRA negative with average percent serotonin release of 2% and 1% in response to low (LH) and high (HH) heparin concentrations, respectively, with 20% release required to indicate platelet activation. The negative SRA sample with the greatest reactivity demonstrated 16% and 8% release to LH and HH, but was immunoassay negative. Conclusions: The association between aPL antibodies and false-positive HIT immunoassays was confirmed in our study, including 2 cases that met criteria for heparin-dependent antibodies. However, no samples demonstrated platelet activation in the SRA test, suggesting that high-titer aPL do not interfere with the SRA assay. The SRA may be the preferred first-line test to evaluate potential HIT in patients with aPL.
A MODEL TO ASSESS COSTS ASSOCIATED WITH THE USE OF FVIII AND FIX ONE-STAGE AND CHROMOGENIC ASSAYS

Steve Kitchen1, James Blakemore2, Kenneth Friedman1, Dan Hart1, Richard H. Ko3, David Perry4, Sean Platton1, Guy Young5, Roger J. Luddington6

1Royal Hallamshire Hospital Sheffield, United Kingdom, 2Cambridge Consultants Cambridge, United Kingdom, 3Blood Center of Wisconsin Milwaukee, WI, USA, 4Barts Health NHS Trust London, United Kingdom, 5Children’s Hospital Los Angeles, University of Southern California Keck School of Medicine Los Angeles, CA, USA, 6Cambridge University Hospital NHS Foundation Trust, Addenbrooke’s Hospital Cambridge, United Kingdom

Introduction: Depending on the reagents and instrumentation used, measurement of coagulation factor activity can be associated with a high level of variability using one-stage activated partial thromboplastin time (aPTT) assays. Chromogenic assays show less variability in the measurement of FVIII and FIX activity levels, but are less commonly used in clinical laboratories. Reluctance among the haematology laboratory community to universally adopt use of chromogenic assays may be partly attributable to a perception of higher associated costs. Methods: Interviews with haematologists and laboratory scientists were conducted to identify key cost parameters associated with the use of aPTT and chromogenic assays. A cost analysis model for FVIII and FIX chromogenic assays relative to aPTT assays was generated based on a series of assumptions related to common clinical laboratory practice, manufacturer list prices (July 2014) and assay kit configurations. Results: Chromogenic FVIII assay costs were similar to aPTT aPTT assays, however FIX chromogenic assays were more costly than FIX aPTT testing. Assay throughput, kit configuration and reagent stability were identified as key cost parameters. Increased laboratory factor assay throughput in the model yielded a cost reduction for chromogenic FVIII assays, but had no impact on the difference between one-stage aPTT and chromogenic FIX assays. Increased batch testing size reduced the cost of aPTT assays, but had limited impact on the cost of chromogenic assays. The use of frozen aliquots of chromogenic kit reagents in the model to counter limited stability at room temperature on the analyser yielded the most significant reduction in cost per test for both FVIII and FIX assays. Conclusions: Chromogenic kit reagent stability on analysers limits the cost efficiency of chromogenic assays relative to aPTT testing. Use of aliquoted, frozen chromogenic kit reagents might improve cost efficiency for both FVIII and FIX chromogenic assays. Education and training with respect to the use of chromogenic assays is required to improve adoption by clinical laboratories.
218 EVALUATION OF A NOVEL, PIONEERING, INTERMEDIATE THROUGHPUT SYSMEX COAGULATION ANALYSER

Irfan Patel, Phil Lane, Ian Mackie, Sam Machin
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Introduction: The inclusion of innovative functionality in new analysers is highly desirable in addition to the prerequisites of sample throughput, result precision and comparability. The Sysmex CS-series of analysers have made significant advances in addressing these issues and the latest analyser continues this trend with determination of platelet function using light transmittance platelet aggregometry, along with clot waveform analysis and mixing studies for inhibitor/lupus anticoagulant determination. We assessed the performance of this new, innovative coagulation analyser (CS-2500, Sysmex Corporation, Japan). Like the CS-2100i, it has the same number of multi-wavelength reaction detectors allowing it to perform clotting, chromogenic and immunoturbidimetric assays, along with platelet aggregation assays. Methods: Performance was compared to the CS-2100i for clotting (prothrombin time, activated partial thromboplastin time, Clauss Fibrinogen, Thrombin Time, Dabigatran assay), chromogenic (Antithrombin, Rivaroxaban) and immunoturbidimetric (D-Dimer) assays. All reagents were supplied by Siemens Diagnostic Healthcare (Marburg, Germany) except for Dabigatran and Rivaroxaban assays which were supplied by Hyphen Biomed (Paris, France). Commercial calibrants and control samples were assessed as well as samples from healthy normal subjects (n=30) and a wide variety of different patients (n=95) (selected to represent the common types of abnormality that might be encountered in the clinical laboratory). Calibration curves were tested once on the CS-2100i and on five consecutive days on the CS-2500. Each control sample was tested 10 times to obtain imprecision data. Results: The CS-2500 showed good linearity and reproducible standard curves, and gave low inter-assay imprecision using commercial normal (CV= 0.33 – 4.13%) and pathological (CV= 0.45 – 4.98%) control samples. Using samples from a range of patients and normal subjects, good correlation was observed between the CS-2500 and CS-2100i in each of the test systems; R²= 0.978 – 0.999, with no clinically significant misclassification, and data points scattered closely around the line of identity. There was no difference between the analysers due to interfering substances (haemolysis, icterus and lipaemia). Sample analysis was 180 PT and 62 PT+APTT samples per hour on the CS-2500, compared to 180 PT and 53 PT+APTT on the CS-2100i. Conclusions: Our results demonstrated that using the CS-2500 analyser, routine coagulation testing and specialised assays can be performed with satisfactory levels of imprecision and showing good correlation with the CS-2100i.

219 COULD ROTEM REPLACE ROUTINE PEDIATRIC ECMO COAGULATION TESTING?

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1Texas Children’s Hospital Houston, TX, USA, 2Baylor College of Medicine Houston, TX, USA

Introduction: The panel of coagulation tests include; prothrombin time (PT), activated partial thromboplastin time (PTT), fibrinogen, Heparzyme PTT (HPTT), antithrombin (AT), anti-Xa assay, and platelet count (PLT) are routinely used in our hospital to monitor hemostatic balance and heparin therapy in the pediatric extracorporeal membrane oxygenation (ECMO) setting. Rotational thromboelastometry (ROTEM) provides a global hemostasis assessment and results of these bedside tests could be evaluated within 10-20 min. The utility of ROTEM was shown in surgery and trauma settings however the utility in ECMO monitoring is unknown. Objectives: The aim of the study is to evaluate ROTEM as an ad-junction or replacement for current coagulation testing in pediatric ECMO patients. Methods: This was a single center IRB-approved prospective study on pediatric ECMO patients. Citrated whole blood (2 mL) was collected for ROTEM analysis while a paired sodium citrate sample was sent to the main lab for coagulation testing. Data collected from ROTEM consisted of INTEM, EXTEM, HEPTEM, and FIBTEM clotting time (CT) and maximum clot firmness (MCF). ECMO coagulation panel was measured on STA-R coagulation analyzer. Collected data was plotted and strength of linearity was assessed using a Pearson Correlation (SPSS 2012) with significance at p< 0.05. Results: Forty five data points from 10 patients (mean, (IQR); age 3.9 years (3.5- 14.6), ECMO length 6 days (3-8), and 3 (2-6) samples per patient were analyzed. Excellent correlation occurred with fibrinogen vs. FIBTEM MCF (r = 0.86 p < 0.001). PLT correlated well with both EXTEM MCF (r = 0.51, p < 0.001) and INTEM MCF (r = 0.51, p < 0.001). Weak, albeit still significant correlation, was found for PTT vs. INTEM CT (r = 0.34, p = 0.01) and PT vs. EXTEM CT (r = 0.32, p = 0.03). HPTT did not correlate with vs. HEPTEM CT (r = -0.01 p = 0.93) as well as AT and anti-Xa activity did not correlate with any of ROTEM test variables. Conclusions: Evaluation of INTEM, EXTEM, and FIBTEM MCF could be used as a good surrogate marker of PLT count and fibrinogen concentration in ECMO setting. CT values of INTEM, EXTEM, and HEPTEM are unreliable to use instead of PTT, PT, and HPTT to monitor heparin therapy or estimate coagulation status in pediatric patients on ECMO. If ROTEM analysis would be useful as an addendum to routine coagulation monitoring in ECMO cases deserves further study.
EVALUATION OF THROMBOTIC RISK IN LUPUS ANTI-COAGULANT POSITIVE PATIENTS; EFFECT OF ANTIPHOSPHOLIPID ANTIBODIES.
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Cleveland Clinic Cleveland, OH, USA

Introduction: Approximately one-third of patients with antiphospholipid antibodies develop thromboembolic events. Lupus anticoagulant (LA) is an independent risk factor for thrombosis. However, association of thrombosis and anticardiolipin antibodies (ACA) and/or beta2-glycoprotein-I antibodies (B2GPI) in LA+ patients remains to be established. This study aims to investigate arterial/venous thrombotic risk in LA+ patients with additional ACA+ and/or B2GPI+.

Methods: Medical records of LA+ patients between 2008 and 2010 were retrospectively reviewed. Clinical history (age/sex, thrombotic events, anticoagulants, and history of known risk factors) and laboratory results (APTT, hexagonal phase phospholipid neutralization (HexPL; Staclot LA), dilute Russell viper venom time (DRVVT; Staclot DRV) ratio and 1:1 mixing, platelet neutralization procedure (PNP), incubated APTT mixing study, ACA, B2GPI, PT/INR, factor VIII, PT gene and factor V Leiden mutation) are reviewed. The diagnostic criteria of LA includes prolonged phospholipid dependent clotting test, evidence of an inhibitor by mixing study, confirmatory test for phospholipid dependent nature and exclusion of specific inhibitors. Statistical methods included Chi-square, Fisher’s exact test, and logistic regression. Odds ratios (OR) with 95% confidence intervals (CI) were calculated.

Results: Among 287 LA+ patients (median 54 years, male/female=1.0), 70.7% patients experienced thrombotic events. Venous thrombosis was more common than arterial thrombosis (53.7%;154/287 vs 28.6%;82/287). Deep venous thrombosis (76%;117/154) and cerebrovascular attack (60%;49/82) were frequent among venous and arterial thrombotic events, respectively. 17.2% of LA+ patients were positive for ACA (IgG 65.3%, IgM 59.2%, IgA 14.3%) and 24% were positive for B2GPI (IgG 52.4%, IgM 61.9%). 13.2% of LA+ patients were positive for both ACA and B2GPI. Figure 1 showed frequency of any arterial/venous thrombotic events in single (LA+), double (LA+ACA+, LA+B2GPI+) and triple (LA+ACA+B2GPI+) positive patients. LA+ patients with ACA had significantly increased risk for any thrombosis compared to single LA+ patients.

<table>
<thead>
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<th></th>
<th>OR</th>
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<td>LA+ACA+</td>
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</tbody>
</table>

LA+ACA+, LA+B2GPI+ and LA+ACA+B2GPI+ patients were associated with arterial thrombosis (OR;95% CI 3.39;1.79-6.48, 2.56;1.22-5.33, 3.81;1.57-9.36, respectively; all p< 0.05), but not venous thrombosis. LA testing was repeated in 43.2% of LA+ patients and 82.3% showed persistent LA+. There was no difference in thrombotic events between persistent LA+ and single LA+ patients (64.7% vs 62.0%, p=0.65). Conclusions: Although this study has a limitation of relatively small numbers, ACA increased the risk of any arterial/venous thrombosis in LA+ patients. Antiphospholipid antibodies in LA+ patients were associated with arterial thrombosis.
Implementation of rotational thromboelastometry (ROTEM) for assessment of trauma-induced coagulopathy in a large trauma centre: experiences from the hematology laboratory perspective
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Vancouver, BC, Canada

Introduction: Rapid diagnosis and treatment of trauma-induced coagulopathy is critical to improve survival rates in trauma. Assessment of coagulopathy can be performed using conventional coagulation tests (CCTs: INR, PTT, Clauss fibrinogen) or by rotational thromboelastometry (ROTEM). The latter provides a global picture of hemostasis by graphically displaying the dynamics of clot development, stabilization and lysis. We implemented ROTEM (Pentapharm GmbH, Munich, Germany) in our central hematology laboratory with the aim of improving the assessment of coagulopathy in trauma patients compared with CCTs without sacrificing turnaround time (TAT). Methods: Vancouver General Hospital is a Level 1 trauma centre. A total of 29 hematology laboratory staff were trained in ROTEM in the month preceding implementation. Between October 8, 2014 and December 31, 2014 all adult patients for whom the trauma surgery team was activated had coagulopathy assessed by both CCTs and ROTEM. The samples were sent immediately to the hematology laboratory via the pneumatic tube system with a coloured flag identifying the ROTEM samples. Additionally, the trauma staff provided telephone notification to the laboratory. Key Extem and Fibtem ROTEM numerical parameters were faxed to the trauma team after both 10 and 30 minutes of an analytic run-time. CCTs were run on ACL TOP 700 (Instrumentation Laboratory, Bedford, MA, USA) and reported in real time via the electronic lab information system. Results: There were a total of 29 trauma patients during the study period. Nine patients (31%) had low Fibrinogen A10 levels (less than 10 mm); of these only one had a low fibrinogen level (less than 1.5 g/L). The median TAT for CCTs was 32 minutes (range 19 – 94 minutes). The median TAT for first ROTEM result was 41 minutes (range 22 – 141 minutes). TAT of ROTEM results showed improvement over time. The primary reason for the delay in ROTEM results compared with CCTs was inadequate communication between trauma team and laboratory. Conclusions: Centralization of ROTEM within a hematology laboratory has the benefit of 24/7 staff experienced in laboratory quality assurance, albeit at a cost of initial delays in result turn around times. For optimal patient management, attention must be paid to promoting excellent communication between trauma staff and the laboratory. Although ROTEM has the potential to better diagnose coagulopathy in trauma patients, discrepancies with conventional coagulation tests raise questions about the applicability of results.

Analysis of CYP2C9 polymorphisms (*2 and *3) in warfarin therapy patients of Pakistan

Introduction: Warfarin is a common anticoagulant known to exert its effect by inhibiting enzyme vitamin K epoxide reductase (VKORC1). Warfarin has narrow therapeutic index and a wide inter individual variability is present in terms of dose of warfarin. CYP2C9 is an enzyme of cytochrome P450 system and involved in metabolism of S-warfarin. CYP2C9 gene shows many single nucleotide polymorphisms (SNP) most commonly found are for 2 alleles CYP2C9*2 (Arg144Cys) located at exon 3 and CYP2C9*3 (Ile359Leu) located at exon 7. These two variant alleles are involved in increased metabolism of warfarin thus cause bleeding tendency. These variants alleles have been studied in different populations. This study defines these two variant alleles frequency in Pakistani population and effect of these polymorphisms on warfarin dose. Methods: Seventy four patients on warfarin therapy after heart valve replacement (HVR) were included in this study. All patients were taking low dose of warfarin (4.90-17.50mg weekly). DNA was separated according to phenol-chloroform method (Smbrook and Russel, 2001). Presence of polymorphism was confirmed by PCR-RFLP technique. Results were confirmed by performing DNA sequencing on selected samples Results: Among 74 patients included in this study 51.3% were females and 48.6% were males. The mean age of patients was 39.0±12.8 years. In our results CYP2C9*2 allele frequency was 12.1%, 17.6% heterozygotes for allele 2 (*1/*2) were present. Homozygosity for allele 2 (*2/*2) was not present in any subject. CYP2C9*3 frequency was found to be 14.6% which was relatively higher than the frequency of this allele other ethnic regions. 21.6% individuals were heterozygous for variant 3 (*1/*3). None of patient was homozygous for *3 (*3/*3). It was observed that warfarin weekly dose was related significantly (p value ≤ 0.001) with variability of genotypes in patients taking long term warfarin therapy. Individuals possessing *3 were found to be associated with low warfarin dose among other genotypes (*1 and *2). Conclusions: It is concluded, that in this cohort polymorphic allelic variants *2 and *3 of CYP2C9 gene are present in high percentage in Pakistani population. High percentage of both variants *2 and *3 suggest significant association with warfarin low dose requirement in patients. In these results, higher percentage of CYP2C9*3 than CYP2C9*2 allele also suggest that *3 allele could be more responsible for the low dose of warfarin in the patients of heart valve replacement (HVR).
A CASE REPORT OF T-LGL LEUKEMIA WITH RARE DOUBLE POSITIVE CD4+/CD8+ IMMUNOPHENOTYPE
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Dalhousie University/CDHA Halifax, NS, Canada

Introduction: T-Cell large granular lymphocyte leukemia (T-LGL leukemia) is a disease characterized by typically indolent clonal proliferation of large granular lymphocytes. This condition is also associated with many clinical and serologic autoimmune findings. The classical immunophenotype of cases of T-LGL Leukemia is CD8+/CD4-CD3+ T-cells. Methods: Here we are reporting a case of T-LGL leukemia where the neoplastic CD3+ T-cells are CD8+/CD4+. Our case is that of a 55 years old male who presented to the outpatient hematology clinic referred from his home hospital for cytopenia not yet diagnosed. The patient has been followed for few months for asymptomatic neutropenia and thrombocytopenia in the absence of anemia. The peripheral blood showed a white blood count of 4.2 x 10^9/L, hemoglobin 154 g/L, neutrophil absolute count of 0.7 x 10^9/L, and platelet count of 93 x 10^9/L. There is no absolute lymphocytosis. However, large granular lymphocytes (LGL) represented ~90% of lymphocytes. The estimated number of LGL cells is > 2 x 10^9/L. The patient underwent a bone marrow aspirate and biopsy. Flow cytometric studies identified a neoplastic T-LGL proliferation with the following immunophenotype: Positive for CD45 (strong), CD7, CD52, CD16, CD57, and HLA-DR; and for CD45 (strong) CD3, CD5 (abnormally weak), CD2, CD4, CD8 (slightly weak), CD7, CD52, CD16, CD57, and HLA-DR; and negative for CD1a, CD56, CD34, CD26, CD25, and CD56. The diagnosis was confirmed by molecular TCR clonality study in a bone marrow sample. Results: This immunophenotype is rare and only few cases are found reported in the literature. Please see figure 1. Conclusions: Our patient remains asymptomatic with gradually worsening neutropenia and thrombocytopenia. He is considered for treatment at the moment in order to control his cytopenia and to avoid its complications T-LGL leukemia. It is not clear at the moment in order to control his cytopenia and to worsen neutropenia and thrombocytopenia. He is considered for treatment at the moment in order to control his cytopenia and to avoid its complications T-LGL leukemia.
311 COMPARISON OF ABBOTT CELL-DYN SAPPHIRE FLOW CYTOMETRY TO BD FACS CALIBUR FLOW CYTOMETRY FOR CD34+ CELL ENUMERATION

Scott Aveillia1, Steven Marionneaux1, Ann Marie Vega1, Virgil Chan1, Wright Don1
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Introduction: The ability to enumerate CD34+ cell content of peripheral blood is crucial to the development and implementation of data directed protocols for optimizing CD34+ hematopoietic progenitor cell (HPC) collections via apheresis methodologies. Traditionally, this enumeration is performed on dedicated flow cytometry platforms, such as the BD FACsCalibur, with user modified sequential gating analysis (as per the ISHAGE protocol). This study evaluated CD34+ cell enumeration on the Abbott CELL-DYN Sapphire hematology analyzer, using the WBC OpenFlow mode. Methods: Peripheral blood samples from HPC donors (n=98) were split for analysis on the BD FACsCalibur and the Abbott CELL-DYN Sapphire. The data obtained on the BD FACsCalibur was analyzed via ISHAGE sequential gating. A sequential gating strategy (SGS) was devised for the Abbott CELL-DYN Sapphire to account for differences in methodologies. Several clinically important CD34+ cells/uL thresholds were examined (5, 10, 15, 20 and 40 CD34+ cells/uL) to determine the truth tables for the Abbott Sapphire/SGS using the BD FACsCalibur/ISHAGE as the reference method. Results: Correlation between the BD FACsCalibur/ISHAGE and Abbott Sapphire/SGS revealed R²=0.96, slope = 0.89 and intercept = 8.19, with data points ranging from 0-400 CD34+ cells/uL.

<table>
<thead>
<tr>
<th>CD34/uL</th>
<th>5</th>
<th>10</th>
<th>15</th>
<th>20</th>
<th>40</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity (%)</td>
<td>99</td>
<td>99</td>
<td>100</td>
<td>100</td>
<td>98</td>
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<tr>
<td>Specificity (%)</td>
<td>67</td>
<td>94</td>
<td>96</td>
<td>89</td>
<td>87</td>
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</table>

Conclusions: Agreement between the Abbott Sapphire and BS FACsCalibur was excellent at various clinically relevant thresholds. Routine hematology analyzers such as the Abbott CELL-DYN Sapphire, with onboard flow cytometry capabilities offer consistent results for peripheral blood CD34+ cell enumeration when compared with traditional flow cytometry. The Abbott analyzer requires less operator intervention, shorter turnaround times for results and does not require technologists with flow cytometry expertise in order to perform the assay.

312 CEREBROSPINAL FLUID BY FLOW CYTOMETRY CHALLENGES AND ADVANCES IN THE DETECTION OF HEMATOLOGIC NEOPLASMS CELLS

Luiz Cameiroa Bento1, Rodolfo Patussi Correia1, Sonia Tsukasa Nozawa1, Ana Carolina Apelle Bortolucci1, Andressa Da Costa Vaz1, Anderson Marega Alexandre1, Daniela Schimidell1, Fabricio Simoes Perini1, Joao Guilherme De Al Franceschi1, Gustavo Bruiniera Per Fernandes1,2, Carlos Augusto Senne Soares1,3, Joao Carlos De Campo Guerra1,2, Marjorie Paris Colombini1, Nydia Strachman Baca1,3
1Albert Einstein Hosp São Paulo, Brazil, 2Centro De Hematologia De São Paulo São Paulo, Brazil, 3Laboratório Senne Liquor São Paulo, Brazil

Introduction: The study of hematologic neoplasms in Cerebrospinal Fluid by conventional morphology, has limited sensitivity. Flow cytometry (FC) has been used as a powerful technique with high sensitivity and specificity for the detection of lymphoma and leukemia infiltration, even when the cellularity is low. In this study, we present the challenges in this technique and the advances to evaluate CSF specimens by flow cytometry. Methods: From January 2014 to December 2014 our group evaluated 49 cases by FC using BD FACS Canto II (Becton Dickinson), DIVA software (Becton Dickinson), and Infincyt (Cytognos). Euroflow Small Sample Tube - SST (CD20V450/CD45V500/CD8FITC+LAMBDAFITC/CD56PE+KAPPAPE/CD4Perp-y5.5/CD19PE-Cy7/CD3APC/CD14APC/CD38APC-H7) was used as screening strategy, and depending on the amount of sample, we extended the phenotypic study according to hematologic malignancy. Results: Our study detected, 20,4% (10/49) of CSF specimens with hematologic malignancies, four cases with B Non-Hodgkin Lymphoma (B-NHL), one B Lymphoblastic Leukemia, one with Blastic Plasmacytoid Dendritic Cell Neoplasm (BPDC) and 4 with Immature Myeloid Population (IMP). The average sample volume was 8,7ml, and even in extremely low cellularity count (B-NHL) we could detect diseases. No phenotypic abnormalities were identified by FC in 79,6% (39/49) of the samples with 7,8ml of volume. Even in samples that have low cellularity and no abnormal cells, we could detect monocytes, CD3 T-lymphocytes, and the subtypes CD3/CD4 and CD3/CD8 in all samples. The great challenge in the investigation of hematologic malignancy in CSF by FC is the low cell count with usual restricted sample volume and increase cell death. In our service, recent advances in technical procedures, equipment, software and fluorochrome have been made to standardize and increase the test sensibility, especially at lower cell concentrations. The improvements were, the migration of 4-colours to 8-colours FC protocols, standardization of technical procedures for better concentration of the samples in one tube, immediate processing of sample (up to 4 hours), analysis in DIVA and Infinity software, and continuous team training. Conclusions: Flow cytometry is a powerful and sensitive technique for cell detection in extremely low count samples, and has become an important tool in improving the diagnosis of hematologic neoplasm in CSF.
LYMPHOCYTE SUBSET ANALYSIS (WITH MEDIAN FLORESCEENCE INTENSITIES OF DIFFERENT ANTIGENTS) ON LYMPHYCYTES IN DENGUE PATIENTS

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BLK Super Speciality Hospital New Delhi, India

Introduction: Infection with any of the Dengue virus (DV) serotypes may be asymptomatic or may result in a wide spectrum of clinical symptoms, ranging from a mild flu-like syndrome to the most severe forms. Cell and tissue tropism of DV may have a major impact on the outcome of dengue infections. However, our understanding of the role played by DV tropism is minimal in absence of animal model studies. Studies suggest that three organ systems play an important role in the pathogenesis of severe manifestation of dengue: the immune system, the liver, and endothelial cell (EC) linings of blood vessels. Within the immune system, antigen-presenting dendritic cells, complement activation, the humoral immune response, and the cell-mediated immune response all are involved in the pathogenesis, leading to activation of T, NK and B cells, which in turn release a cascade of cytokines resulting into the events thereof. We wanted to look at the predominant lymphocyte subsets, their ratios and fluorescence intensities of different antigens on T, B and NK cells, and any additional cells like circulating plasma cells vis-à-vis the “normal” unaffected population. Methods: A total of 32 patients, Dengue NS1 Antigen positive on MacELISA were selected for the study. For comparison blood samples for control group were taken from randomly selected 6 healthy normal people visiting hospital for regular health checkups. Following tests were done on all the subjects of the study: Complete Blood Count (CBC) using Beckman Couter LH-750 analyser, Flow cytometric immunophenotyping of the lymphocytes using FACS Calibur using following antibodies: CD45, CD3, CD5, CD7, CD4, CD8, CD19, CD56, CD38. Cytoplasmic kappa and lambda was done in cases which showed bright CD38 positive plasma cells. All blood specimens were collected in K2EDTA vacutainers. Sample were run within 4 hrs of collection. Results: Following results obtained on the dengue and normal patients are shown in the figure 1 below:

Conclusions: T lymphocyte subset was the predominant population. Viral infections are generally associated with reversal of CD4:CD8 ratio, however, in our study we found that 27 of 32 dengue patients had a CD4:CD8 ratio of >1. Interestingly, 15 of 32 dengue cases had circulating plasma cells which were polyclonal as confirmed by cytoplasmic kappa and lambda. Also, the median fluorescence intensities of CD45, CD3, CD7, CD4 and CD8 on T cells, CD19 on B cells and CD56 on NK cells were significantly lower on the T, B and NK Cells in dengue patients when compared with the normal controls, indicating their release in the peripheral blood at relatively immature level. While more conclusive research with an increasing footprint of samples is still in the future, our study demonstrates distinct reduction in fluorescence intensities with maintenance of CD4:CD8 ratio.

ABNORMALITIES IN LYMPHOCYTE SUBSETS AND THEIR IMMUNOPROFILE IN PATIENTS WITH CHRONIC EOSINOPHILIA INCLUDING LYMPHOCYTIC VARIANT OF HYPEREOSINOPHILIC SYNDROME AND IGG4-RELATED DISEASE

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Introduction: Chronic Eosinophilia (CE) is a heterogeneous group of disorders with diverse pathogenesis, variable clinical and laboratory presentation, clinical course, and equally varied treatment. Recently abnormal T/NK populations in certain subgroups of CE namely lymphocytic variant of hypereosinophilic syndrome (L-HES) and IgG4-related disease (IgG4-RD) have been described. Occurrence of such abnormal T/NK-cell populations in unselected CE disorders has not been reported. Methods: Raw flow cytometry data of 80 cases of T-cell immunophenotyping of 34 patients with CE were reanalyzed. Blood and/or bone marrow specimens were stained with antibodies to one or more of CD2, CD3, CD4, CD5, CD7, CD8, CD10, CD16, CD19, CD20, CD56 and CD45, analyzed with Canto II flow cytometer and the data interpreted with a standard template using FACS Diva software, by four investigators. In addition to the usual T/NK parameters, number of CD3+/4+, CD7-/4+, CD3+/4-/8+, and CD3+/8+/56+ cells and their detailed immunoprofile was recorded. Results: One or more of the above-mentioned T-NK abnormalities were seen in 27 instances in 21/34 patients. Specifically, expanded populations of CD3+/4+, CD56+ CD3+/4-/8+, CD3+8+/56+ and NK cells were seen in 6, 9, 7, 2 and 3 patients respectively. Clinical and Laboratory correlations of these immunophenotypic abnormalities will be reported. Conclusions: Diverse abnormalities of T-NK populations as described above are frequently seen in patients with CE, including L-HES, and IgG4-RD. At least some of them affect subpopulations known to secrete eosinopoietic cytokines.
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LEUKAEMIA-ASSOCIATED IMMUNOPHENOTYPES IN NORMAL BONE MARROW BLASTS: RELEVANCE IN ACUTE MYELOID LEUKAEMIA MINIMAL RESIDUAL DISEASE ASSESSMENT.
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1North Shore Hospital Auckland, New Zealand, 2Haematology Laboratory, Auckland City Hospital Auckland, New Zealand

Introduction: Minimal residual disease (MRD) status of patients undergoing treatment for acute myeloid leukaemia (AML) is important for prognosis and guiding further treatment decisions. Multicolour flow cytometry (MCF) is a sensitive method for MRD assessment. The current approach relies on the identification of blasts expressing Leukaemia-Associated Immunophenotypes (LAIP). Expression of LAIP on normal myeloblasts however affects the specificity of the result. There are only limited published data available in this regard. We report our findings from 14 normal adult bone marrows.

Methods: Flow cytometry analysis was performed on the residual normal marrow specimens from 14 adult patients after the intended investigations were completed. The expressions of CD15, CD11b, CD7, CD4, and CD56 on CD34+ myeloblasts were assessed. LAIP is defined by lineage infidelity or asynchronous expression of differentiation markers.

Results: Myeloblasts constituted 1.136% (range 0.137-2.800%) of CD45 cells in the marrow. Percentages with LAIP are shown in the tables. Table 1. Percent of normal myeloblasts with LAIP

<table>
<thead>
<tr>
<th>CD15</th>
<th>CD11b</th>
<th>CD4</th>
<th>CD7</th>
<th>CD56</th>
</tr>
</thead>
<tbody>
<tr>
<td>14/14 (100%)</td>
<td>13/14 (93%)</td>
<td>10/14 (71%)</td>
<td>14/14 (100%)</td>
<td>10/14 (71%)</td>
</tr>
<tr>
<td>18.36%</td>
<td>1.65%</td>
<td>1.13%</td>
<td>9.93%</td>
<td>1.61%</td>
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<tr>
<td>19.88%</td>
<td>1.21%</td>
<td>1.04%</td>
<td>8.13%</td>
<td>1.52%</td>
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<tr>
<td>6.75-25.15%</td>
<td>0.16-4.33%</td>
<td>0.72-2.00%</td>
<td>3.38-23.78%</td>
<td>0.45-3.32%</td>
</tr>
</tbody>
</table>

Table 2. Normal CD34+ myeloblasts with LAIP as percent of CD45+ cells

<table>
<thead>
<tr>
<th>CD15+</th>
<th>CD11b+</th>
<th>CD4+</th>
<th>CD7+</th>
<th>CD56+</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.199%</td>
<td>0.016%</td>
<td>0.011%</td>
<td>0.026%</td>
<td>0.019%</td>
</tr>
<tr>
<td>0.030-0.442%</td>
<td>0.001-0.043%</td>
<td>0.081-0.027%</td>
<td>0.019-0.260%</td>
<td>0.001-0.045%</td>
</tr>
<tr>
<td>14/14 (100%)</td>
<td>13/14 (93%)</td>
<td>10/14 (71%)</td>
<td>14/14 (100%)</td>
<td>10/14 (71%)</td>
</tr>
</tbody>
</table>

Conclusions: The percentages of normal myeloblasts with LAIP involving the markers used and are above the MCF detection sensitivity of 0.01% varies between 43 to 100%. This limits the specificity of the results for AML MRD. Even if the threshold is raised to 0.1%, there will still be false positive cases using aberrant CD15 or CD7, which will impact on patient management. Our work provided diagnostic information for AML MRD determination in our laboratory. A database of LAIP on normal myeloblasts is required to determine the optimal diagnostic cut-off for AML MRD using LAIP. This can be institute specific or through inter-institution collaboration.

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FOUR-COLOR FLOW CYTOMETRIC ANALYSIS FOR QUANTIFICATION OF BLAST CELLS IN BONE MARROW
Seon Joo Kang, Kyung Ho Choi, Sung Ran Cho
Ajou University School of Medicine Suwon, South Korea

Introduction: Flow cytometry that targets specific surface or intracellular antigens is a powerful discriminative tool for diagnosing hematologic malignancy. Its sensitivity is higher than those of standard morphologic review or immunohistochemistry. This study evaluated a 4-color immunophenotyping panel for blast cell quantification in bone marrow.

Methods: Blast counts of Wright-Giemsa stained smears on bone marrow aspirates from 66 patients (43 males and 23 females; mean age, 49 ± 25 yrs) were compared to the percentage of blast cells obtained with 4-color methods on a Cytomics FC500 (Beckman Coulter Inc., USA) with CXP software (Beckman Coulter Inc.). Four monoclonal antibodies were used for flow cytometric analysis of blasts: phycoerythrin-cyanin (PC) 7-conjugated CD45; PC 5-conjugated CD34; phycoerythrin-conjugated CD117; and fluorescein isothiocyanate-conjugated HLA-DR. Data were compared with the Pearson correlation and Wilcoxon signed rank test. Cohen’s kappa coefficient was used to assess agreement.

Results: The blast cells ranged from 0% to 99% by smear and from 0.1% to 89.9% by flow cytometer, respectively. Flow cytometric quantification showed a strong correlation with morphologic count (r²=0.85, P<0.001), and no significant difference in blast cell percentages was found (P=0.940). When the results were categorized by smear blast percentage into groups I (blast< 5%, n=43), II (5%≤blast< 20%, n=8), and III (blast≥20%, n=15), substantial agreement was found between the measurements (kappa = 0.611) and the concordance rate was 80%. However, correlation between morphologic blast count and flow cytometric blast count was poor in group I (r²=0.37, P<0.001) and statistically insignificant in group II (r²=0.03, P=0.704).

Conclusions: Four-color flow cytometric immunophenotyping may be a useful supplementary approach for the objective and precise quantification of blast cells, especially in acute leukemia. Further evaluation of a large number of cases is required.
EXPRESSION OF CD154 ON STIMULATED T CELLS IN PATIENTS WITH IDIOPATHIC THROMBOCYTOPENIC PURPURA
Jimmung Kim, Ikchan Song, Kyechul Kwon, Suhhoe Koo
Chungnam National Hospital Daejeon, South Korea

Introduction: The CD40-CD154 interaction are thought to play a key role in T cell dependent humoral immune response. Increased expression of CD154 on stimulated T cells is observed in rheumatic diseases and is associated with disease activity. With regard to idiopathic thrombocytopenic purpura (ITP), glycoprotein IIb/IIIa-reactive CD4+ T cells are involved in the pathogenesis. In this study, we investigated the expression of CD154 on T cells from ITP patients in comparison with healthy controls and assessed the significance of CD154 expression in disease status. Methods: We enrolled 68 ITP patients (20 men, 48 women, age 55.3±18.8 yr) and 23 healthy controls (10 men, 13 women, age 55.1±17.9 yr). Patients did not receive therapy for at least 2 weeks prior to analysis. Heparinized blood samples were obtained and mononuclear cells (MCs) were isolated by density gradient centrifugation. MCs were stimulated in RPMI medium containing PMA (5 ng/mL) and ionomycin (500 ng/mL) for 2h at 37°C. The expressions of CD154 on CD4+ T cells were measured using flow cytometry. Results: In ITP patients, the percentage of positive CD154 on activated CD4+ T cells was not different compared to that of healthy controls (62.7±11.5% vs 60.0±13.8%). Mean fluorescence intensity (MFI) of CD154 on CD4+ T cells was not different compared to that of healthy controls (3.19±0.64 vs 2.87±0.82, P=0.11). According to the platelet counts, the percentage (64.3±9.9% vs 60.9±9.6%, P=0.17) and MFI of CD154 (3.34±0.63 vs 3.09±0.59, P=0.10) on CD4+CD154+ population showed no difference between 35 ITP patients with less than 50,000/μL and 33 ITP patients with 50,000/μL or more. Conclusions: Increased CD154 expression on CD4+ T cells is not observed in ITP patients and is not related with low platelet counts. Overexpression of CD154 on CD4+ T cells is unlikely to be a central pathogenetic defect in ITP and other immune dysfunctions should be considered as therapy targets.

THE USEFULNESS OF WHITE PRECURSOR CELL (WPC) CHANNEL ON SYSMEX XN-2000 ANALYZER TO DIFFERENTIATE BLAST CELL LINEAGE IN ACUTE LEUKEMIA: A PRELIMINARY STUDY
Agus Susanto Kosasih1, Lyana Setiawan1, Christine Sugianto1, Rudianto2
1Dharmais National Cancer Hospital Jakarta, Indonesia, 2Anna Medika Hospital Bekasi, Indonesia

Introduction: Acute leukemia is one of the leading malignancy and cause of death by cancer. Prevalence of acute leukemia in Indonesia is increasing and demanding a quick detection and management. The application of WPC channel as additional criteria to enable more specific flagging had been reported to be useful in previous studies. In this study we aimed to evaluate whether there is a specific pattern of flagging and scattergram using WPC channel that can differentiate between acute leukemia of different lineage. Methods: The specimens were 28 peripheral blood and bone marrow specimens from patients with acute leukemia referred to the Clinical Pathology Laboratory of Dharmais National Cancer Hospital (DNCH) for hematology assessment and/or immunophenotyping were analyzed using XN-2000. The flagging were then compared to the flowcytometric evaluation. Four clinical pathologists then analyzed the WPC scattergram patterns independently without knowing the diagnosis, and evaluate the lineage based on the patterns. The results were then compared with immunophenotype. Results: Of 28 specimens, 27 (96.4%) showed positive blast flag. One specimen that did not showed blast flag showed abnormal lymphocyte flag with mixed B/T phenotype. Of 13 acute myeloid leukemia, all showed positive blast flag, 10 (76,9%) with positive immature granulocyte flag, and 3 (23,1%) with abnormal and/or atypical lymphocyte flag. Eight (72.7%) out of 11 B-acute lymphocytic leukemia samples showed abnormal and/or atypical lymphocyte flag. The samples from T-lineage acute lymphocytic leukemia patients did not show any specific pattern, might be due to too few cases (n=3). The concordance between interpretation by scattergram and immunophenotype varied between 53.6% to 82.1%. Eleven (39%) cases were correctly interpreted by all observers. Two (7%) cases were wrongly interpreted by scattergram by all observers, 4 (14%) cases by 3 out 4 observers, 3 (11%) cases by 2 out of 4 observers and 8 (29%) by 1 out of 4 observers. Of the 6 cases that were poorly interpreted, 4 cases have leucocyte count below 7.7 x 10^9/L, one case was a case of mixed phenotype B/Myeloid and the other was myelomonocytic leukemia. Conclusions: Combination of flags and scattergrams generated by WPC channel can improve interpretation of acute leukemia lineage. A study with more samples may be required.
IMMUNOPHENOTYPIC ANALYSIS OF HAEMATOLOGICAL MALIGNANCIES IN PAKISTANI POPULATION

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Aga Khan University Karachi, Pakistan

Introduction: Immunophenotyping has become an essential tool for diagnosis and characterization of hematological malignancies. It plays a critical role in diagnosis, classification, prognosis and detection of minimal residual disease. The objective of this study was to see the frequency of Hematological malignancies diagnosed via immunophenotyping in our population. Methods: Material & Methods: Retrospective collection of data on a specially designed Proforma from consecutive cases of Hematological malignancies referred to department of Pathology Aga Khan University Karachi between the years 2009 to 2010. Results: A total of 210 patients aged between 3 to 80 years who had been diagnosed with malignant hematological disorders were included in the study. Among them 66.6% were males (n = 140) and 33.4% females (n = 70). The overall median age in adults at diagnosis was 42 years. Acute myeloid leukemia was most frequent (34.7%) with a median age of 32 years, followed by chronic lymphocytic leukemia with 22.2% (median age 60 years), Non-Hodgkin lymphoma (19.9%; median age 40 years), Acute Lymphoblastic Leukemia (13.6%; median age 25 years), Hogdkin’s lymphoma (5.9%; median age 36 years) and Hairy cell leukemia (3.7%; median age 60 years). Below the age of 20 years, acute lymphoblastic leukemia was predominant (65.7%), followed by acute myeloid leukemia (34.3%). Conclusions: This study shows the pattern of Hematological cancers in our population. There might be under-reporting of Hematological cancers in women. Further studies are required to find out prevalence and distribution of these malignancies.

CD126 EXPRESSION IN MYELOMA PRECURSOR DISEASE AND THE EFFECT OF BONE MARROW ENVIRONMENT

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Flow Cytometry Laboratory, Laboratory of Pathology, CCR, NCI, NIH Bethesda, MD, USA, 2Biostatistics and Data Management Section, CCR, NCI, NIH Bethesda, MD, USA, 3Myeloma Service, Memorial Sloan-Kettering Cancer Center New York, NY, USA, 4Lymphoid Malignancies Branch, CCR, NCI, NIH Bethesda, MD, USA

Introduction: CD126 is a subunit of IL-6 receptor which upon interaction with IL6, plays a vital role in myeloma cell proliferation, and disease progression. It is unclear whether malignant plasma cells have innate elevated CD126 expression or if this is secondary to bone marrow (BM) environment. To further clarify the role of CD126 expression level in plasma cell disease (PCD), CD126 was quantitated in neoplastic and normal plasma cells (nPCs) within BM aspirates (BMA) from patients with monoclonal gammopathy of undetermined significance (MGUS) and smoldering multiple myeloma (SMM), where both populations are exposed to the same BM environment. Methods: 80 BMA from MGUS and SMM patients in addition to 4 BMA from patients who were determined to not have a PCD were evaluated. The diagnosis of PCD was based on clinical findings, serum monoclonal protein, BM morphology and immunohistochemistry, and BMA flow cytometry. The patients were categorized as having MGUS or SSM based on the International Myeloma Working Group criteria. According to Mayo Clinic model, MGUS and SMM patients were assigned into different risk categories for progression to multiple myeloma (MM). CD126 expression was determined on both nPCs and abnormal plasma cells (aPCs) using the QuantiBRITE system (BD Biosciences) to detect the antibody binding capacity (ABC). Results: The median nPCs CD126ABC was lower in BMA with no PCD (538, N=4) than BMA with PCD (791, N=30) but the difference was not significant. The lack of a significant difference in CD126 expression in nPCs and aPCs in all patients (r=0.34, p=0.048). Interestingly, there was weak evidence that CD126 expression by aPCs was higher than that by nPCs in the SMM patients (p=0.048) but not MGUS patients. There was no significant difference in aPCs CD126ABC levels between the three Mayo MGUS risk groups but the SMM Mayo risk group 3 had lower aPCs CD126ABC than the Mayo risk groups 1 and 2 (both p=0.019). Conclusions: The lack of a significant difference in CD126 between aPCs and nPCs and the correlation between nPCs and aPCs in same specimen suggests that the BM environment in MGUS and SMM may be an important determinant of CD126 expression in the neoplastic cell. The observation that the correlation between the CD126ABC in the aPCs and the nPCs decreased in SMM as the Mayo risk increased suggests there may be decreased control of CD126 expression by the BM environment as there is neoplastic progression.
FLOW CYTOMETRY CONFIRMATION AND QUANTIFICATION OF FETOMATERNAL HEMORRHAGE FOLLOWING POSITIVE SCREENING: A SINGLE CENTER EXPERIENCE
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Introduction: The detection of fetal red blood cells in the maternal circulation is important for assessing fetomaternal hemorrhage and determining the dose of immune globulin for Rh prophylaxis. Flow cytometry is preferred for its consistency and sensitivity to the microscopic methods; but its application is limited by the relative complexity and operation cost. Methods: We have used flow cytometry for confirmation and quantification following the manual Kleihauer-Betke (KB) test and Rosette anti-D screening. This study evaluated results from a total of 2308 patients who were tested for possible fetomaternal hemorrhage during a 2-year period. Results: Of the 2308 patients, 155 (6.7%) had initial positive KB or anti-D Rosette and underwent flow cytometry analysis. The flow cytometry detection limit was set at 0.05% based on commercial controls and reference values of fetal red cells in maternal blood. Of the 155 patients, 69 (45%) had measurable level (>0.05%) of fetal RBCs, and only 7 of these patients had over 30 ml fetal bleed, including 5 patients with more than 100 ml; 86 patients (55%), including 4 Rh-negative patients with positive anti-D Rosette, were counted as negative by flow cytometry (< 0.05% and < 2.5 ml). Flow cytometry showed higher numbers of maternal F-cells in the negative group than that in the positive group (0.6-32.8%, median 10.3% vs. 0.5-19.7%, median 3.4%), which may be related to the false KB positive screenings. Conclusions: Our results indicate that microscopic screening may exclude over 90% cases for flow cytometry analysis for fetomaternal hemorrhage; furthermore, only 4.5% of the positive screenings had significant fetal bleed that require more than standard dose of RhD immune globulin. Flow cytometry in combination with simple and quick screening is an effective and appropriate approach to assess fetomaternal hemorrhage.

CD116 IS A POTENTIALLY USEFUL MARKER IN THE DIAGNOSIS AND MRD DETECTION IN ACUTE MYELOID AND MONOCYTIC LEUKEMIA
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Introduction: CD116 is a GM-CSF receptor, prominently expressed on myeloid cells. Its expression in different hematopoietic cells and its use in diagnosis and monitoring of acute leukemia (AL) is poorly studied. We studied the expression of CD116 in different normal & leukemic hematopoietic cells and its potential use for diagnosis and MRD detection in acute myeloid leukemia (AML). Methods: 128 specimens (41 blood and 87 BM) from healthy donors, uninvolved staging BM, AL patients (AML M0, M1, M2, M4, M5 and ALL) and non-leukemic myeloblasts in post-induction BM from B-ALL patients were studied using 6-8 color immunophenotyping. Levels of CD116 expression were determined as calculating mean fluorescent intensity (MFI). We studied its use in differentiating leukemic myeloblasts from post-induction non-leukemic myeloblasts and hence its role as a potential AML-MRD marker. Results: Figure-1 elaborates the expression of CD116. It was negative (MFI < 2) in lymphocytes, leukemic lymphoblasts and post-induction non-leukemic myeloblasts. CD116 was strongly expressed on mature monocyes followed by immature monocyes, mature granulocytes (neutrophils), and granulocytic precursors. Its sensitivity in differentiating myeloblasts in AML with monocytic differentiation (M4 and M5) from lymphoid blasts (ALL) was 90.32% and AML (M0, M1, M2) was 63.33% and specificity was 100%. Notably, its sensitivity & specificity in differentiating myeloblasts from post-induction non-leukemic myeloblasts was 74.58 % & 100 % respectively. Conclusions: CD116 is strongly expressed in monocytic cells. It is a sensitive and highly specific marker in diagnosing myeloid monocytic leukemia. It can be potentially useful in MRD determination in AML.

<table>
<thead>
<tr>
<th>Type of cells</th>
<th>Sample Type</th>
<th>Sample Number</th>
<th>Mean MFI</th>
<th>CD116 MFI</th>
<th>CD116 Range</th>
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<td>Lymphocytes</td>
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<td>4.14</td>
<td>1.08-19.11</td>
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<tr>
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<td>1.13</td>
<td>1.02</td>
<td>0.27-3.18</td>
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<td>1-40</td>
<td>0.08</td>
<td>0.08</td>
<td>0.00-1.13</td>
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<tr>
<td>Mature Granulocytes (myeloblasts)</td>
<td>Negative BM</td>
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<td>0.67</td>
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<td>Normal Myeloblasts</td>
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<td>AML M4/M5/Blasts</td>
<td>Normal BM</td>
<td>1-40</td>
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<td>AML M4/M5/Blasts</td>
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<td>0.56</td>
<td>0.56</td>
<td>0.00-1.42</td>
</tr>
</tbody>
</table>

*Sample types: ALL = acute lymphoblastic leukemia; BM = bone marrow leukemia; KB = Kleihauer-Betke; Rosette anti-D = anti-D Rosette; PB = peripheral blood. MFI = mean fluorescent intensity.*
410 PREVALENCE AND CLINICAL IMPACT OF IDH1 AND IDH2 MUTATIONS AMONG CYTOGENETICALLY NORMAL EGYPTIAN ACUTE MYELOID LEUKEMIA PATIENTS

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Introduction: This study aimed to evaluate the clinical impact and prognostic relevance of the presence of IDH1 and IDH2 mutations among cyogenetically normal AML patients. Methods: Two hundred and eleven AML patients were included in this study. DNA was extracted from bone marrow samples at diagnosis. The exon 4 of IDH1 and IDH2 were amplified by polymerase chain reaction (PCR) and then sequenced for detection of mutations. Concurrent mutations in NPM1, FLT3, were detected only in AML with the IDH1 and or IDH2 mutations. Results: IDH1 mutations were detected in 18 out of 211 AML patients (8.5 %) in the form of 8 cases R132H, 6 cases R132C, 2 cases R132S, 1 case R132G and 1 case R132V mutation. IDH2 mutations were detected in 22 out of 211 AML patients (10.4 %) in the form of 20 cases R140Q and 2 cases R172K. The IDH1 mutations were detected more frequently in the older age, in female than male, in cases with high platelets count and high bone marrow blast cells; but not related to French-American-British subtypes, performance status, extramedullary disease, hemoglobin concentration. Furthermore; the IDH2 mutations were not related to sex, French-American-British subtypes, performance status, extramedullary disease, hemoglobin concentration, bone marrow blast cells count. On the other hand; the IDH2 mutations were common in older age, associated with low WBC count and high platelets count. In patients with CN-AML with IDH1 and IDH2 mutations with NPM1 mutations without FLT3-ITD adversely impacted overall survival (P = 0.02). Conclusions: Overall, 18.9% of newly diagnosed AML patients with normal karyotypes had alterations of IDH1 genes. AML patients with IDH genes mutations and with NPM1 mutation had bad prognosis. Molecular testing for IDH1 and IDH2 mutations at diagnosis could refine stratification of AML patients with normal cytogenetic

411 DONOR CHIMERISM KINETICS ANALYSIS TO ASSESS ACUTE LEUKEMIA RELAPSE IN ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION.

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First Pavlov State Medical University of St.Petersburg, Russia

Introduction: Allogeneic hematopoietic stem cell transplantation is an effective treatment for different hematological malignances. Efficiency of donor cell engraftment is determined by donor chimerism evaluation, i.e. determining of genetically different cell populations coexistence in patient. We assume that the kinetics of chimerism in patients with hematologic malignancies, in turn, may be defined in terms of relapse probability, and it could be used as a marker of minimal residual disease (MRD) after allogeneic transplantation of HSCs (allo-HSCT). Methods: We have analyzed 440 allo-HSCT’s in patients with various hematologic malignancies. For the donor chimerism monitoring we used standard panels of human DNA markers based on STR variability (short tandem repeats, microsatellites). The ratios of donor and recipient cells were calculated according to the data derived from fragment analysis, using the Genemarker program. The study was performed on days +15, +30, +45, +60 and +100 after transplantation. Results: Achieving of full donor chimerism (95%) significantly increases the 5-year survival (p = 0.001) and reduces the probability of disease recurrence (p = 0.01). Among patients with mixed chimerism the stability of donor chimerism level is most important, being associated with lower risk of relapse (p = 0.013). Thus, in patients with stable mixed chimerism, the markers molecular markers of MRD had no significant effect either on overall survival, or the likelihood of relapse (p = 0.55 and p = 0.34 respectively). Meanwhile, we had found an inverse correlation between the level of donor chimerism and expression of relapse markers, i.e. WT1 (R = 0.4, p = 0.0001) and EVI1 (R = -0.4, p = 0.03). Similar data were obtained when analyzing expression of the chimeric transcripts; BCR-ABL (R = -0.26, p = 0.0001), MLL-AF4 (R = -0.36, p = 0.003), TEL-AML (R = -0.37, p = 0.003). However, this dependence with other markers (CBFB-MYH11, PML-RARα, RUNX1-RUNX1T1) could be also obtained. Conclusions: Monitoring of donor chimerism after allo-HSCT adequately reflects the dynamics of donor cells engraftment and may be used as an adequate method for predicting recurrence of the disease, but it can not be used as a single method in MRD analysis.
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**NEUREGULIN-1 INCREASES SURVIVAL BY REDUCING LEUKOCYTE ACCUMULATION, APOPTOSIS AND INFLAMMATION IN MICE WITH EXPERIMENTAL CEREBRAL MALARIA (ECM)**

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1University Of Ghana Medical School Accra, Ghana, 2Morehouse School of Medicine Atlanta USA, 3Morehouse School of Medicine Atlanta USA, 4Morehouse School of Medicine Atlanta USA, 5Morehouse School of Medicine Atlanta USA

**Introduction:** Cerebral Malaria (CM) is a diffuse encephalopathy caused by infection with Plasmodium falciparum. CM disproportionately affects young children under five and is a major cause of malaria-related mortality. Despite availability of antimalarial drugs, CM-associated mortality remains high at 30% and a subset of survivors may experience short- or long-term cognitive disabilities and other neurological sequelae. Thus, adjunctive therapy is greatly needed to protect against the adverse effects of CM pathogenesis including CNS injury and CM-associated mortality. Neurotrophic growth factor, Neuregulin-1 (NRG-1), protects against neuroinflammation and brain injury associated with acute ischemic stroke (AIS) and acute neurotoxin exposure via anti-inflammatory regulation. Since CM shares features of pathogenesis common to AIS such as vascular congestion, focal inflammation, blood-brain barrier (BBB) dysfunction and apoptotic activation leading to permanent or reversible neuronal damage, we hypothesized that intravenous or intra-peritoneal delivery of NRG-1 can protect against CM severity and associated mortality.

**Methods:** We tested the combined effects of NRG-1 and a standard anti-malarial drug artemether on experimental cerebral malaria (ECM)-induced inflammation and mortality in P. berghei ANKA (Pha)-infected mice. NRG-1 (1.25ng/kg/day) alone from day 6-9 reduced mortality by 73% compared to saline-treated controls. However, combination treatment with NRG-1 and ARM (25mg/kg/day) from day 6-9 post infection improved survival in mice with ECM to 91%. Remarkably, combined therapy reduced systemic and brain pro-inflammatory factors TNFa, IL-6 and IL-1α and enhanced anti-inflammatory factors while decreasing leukocyte accumulation in brain microvessels.

**Results:** Additionally, NRG-1 regulates host immune response cytokines associated with CM severity and CM fatality including CXCL10, IFNy and IL-1β. Interestingly, neuroprotective cytokines, IL-15 and IL-7 were up-regulated by NRG-1 treatment suggesting these cytokines are involved in protection against CM pathogenesis. Conclusions: Neuregulin-1 treatment increases CM survival by 73% in late-stage experimental cerebral malaria (ECM). Combination of NRG-1 with antimalarial improves ECM survival to 91%. Host immune response is modulated in P. infected mice by NRG-1 treatment which also increases expression of IL-15 (neuroprotective factor) and IL-7 (neuronal growth factor). Neuregulin-1 attenuates leukocyte accumulation within brain microvessels in mice with ECM. The protective effect of neuregulin-1 in ameliorating late stage ECM indicates a potential for use as adjunct therapy coupled with current anti-malarials. This may provide a new therapeutic approach for cerebral malaria management. We conclude that NRG-1 protects against CM pathogenesis, and thus, may represent novel adjunct therapy for the treatment of CM-associated neurological disorders and reduce CM related mortalities.

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**PLASMODIUM FALCIPARUM APOPTOTIC FACTORS INDUCE APOPTOSIS IN BRAIN VASCULAR AND HEMATOPOIETIC STEM CELLS**

Carmen Dickinson Copeland, Felix Botchway, Nana Wilson, Mingli liu, Vincent Bond

1Morehouse School of Medicine Atlanta, GA, USA, 2University of Ghana Medical School Accra Ghana, 3Morehouse School of Medicine Atlanta, GA, USA, 4Morehouse School of Medicine Atlanta, GA, USA, 5Morehouse School of Medicine Atlanta, GA, USA

**Introduction:** The severity of malaria caused by both Plasmodium falciparum and P. bergheri-ANKA is determined by many elements, including parasite and host apoptotic and inflammatory factors such as Plasmodium apoptotic factor (PAF), Heme, CXCL10 and others. Soluble apoptotic factors that induce apoptosis in both mouse and human brain microvascular endothelial cells (MBVEC & HBVEC) and neuronal cells have recently been identified. Additionally, early endothelial progenitor cells such as hematopoietic stem cells (HSC) are depleted in humans with cerebral malaria (CM) probably due to apoptosis or stasis in these cell types. The mechanism(s) by which these cells are targeted, activated or depleted is unclear. Recent studies indicate a novel role of parasite- or host-derived microparticles (Mp) in dissemination of these factors leading to severity of malaria.

**Methods:** MBVEC, HBVEC and HSC viability was assessed using MTT assay and apoptotic indices were measured using TUNEL assay and induced caspase-3-expression. Significant decreases (p< 0.05) in viability and increases (p< 0.05) in apoptosis were observed in PAFs, Hemin and Mp-treated MBVEC, HBVEC and HSC versus non-treated controls.

**Results:** Plasmodium apoptotic factors play an important role in inducing apoptosis in MBVEC, HBVEC and HSC. The depletion of cEPC contributes to the serious complication of malaria. Plasmodium apoptotic factors could be novel therapeutic targets for severe malaria through decreasing BBB damage and preventing HSC depletion.

**Conclusions:** PAFs, Hemin and Malaria (+) MP decreased viability and increased apoptosis at IC₅₀ in MBVEC, HBVEC and CD34+ HSC when compared to non Malaria (-) MP, indicating that these factors may be deleterious to host endothelium and integrity of the BBB. Hemin and Malaria (+) MP increased apoptosis at IC₅₀ in HBVEC and CD34+ HSC as indicated by increased Caspase3 expression, in agreement with TUNEL positivity assays. PAFs, although induce DNA fragmentation, did not significantly induce Caspase3 expression, indicating that PAFs may be deleterious to host endothelium and integrity of the BBB through an alternative mechanism. Plasmodium induced and host derived apoptotic factors could be novel therapeutic targets for severe malaria.
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HAEMOPHILIA A CARRIER DETECTION IN SUDANESE FAMILIES: DNA LINKAGE ANALYSIS APPROACHES
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Introduction: Haemophilia A is the most common X-linked inherited bleeding disorder caused by a deficiency in the activity of coagulation factor VIII, with an incidence of 1 in 5000 male births. Genetic diagnosis of Haemophilia A is the most accurate method available for carrier detection. Direct mutation detection for haemophilia A is difficult and expensive, accordingly genetic testing for carrier detection has relied upon indirect linkage studies. So this study aimed to investigate the usefulness of FVIII intron 18 BclI RFLP, intron 13 and intron 22 CA repeats linkage analysis for carrier detection in Sudanese families. Methods: This was a prospective, cross sectional, analytical and community-based study. Following written informed consent 20 families with at least one subject affected with Haemophilia A, and 30 unrelated normal females as control group were enrolled. PCR and restriction enzyme analysis were used to study the polymorphism in BclI. Intron 13 and intron 22 CA repeats were analyzed using fluorescent PCR followed by capillary electrophoresis. Results: The incidence of BclI (+) allele was 78%, 39.5% and 33% in patients, female relatives and control group respectively. Expected heterozygosity for BclI was 0.48 in female relatives compared with 0.46 in the female control group. However, observed heterozygosity was found to be 0.54 in female relatives compared to 0.66 in the female control group. The defective X chromosome could be tracked down in 4/9 (44.4%) mothers. Conclusions: PCR-RFLP using BclI is informative in carrier detection of Haemophilia A in the Sudanese population. BclI is more informative compared to both Intron 13 and Intron 22 CA repeats.

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THE INFLUENCE OF METHYLENETETRAHYDROFOLATE REDUCTASE (MTHFR) POLYMORPHISMS ON THE SIDE EFFECTS OF MEDIUM DOSE METHOTREXATE INFUSION IN CHILDREN WITH ACUTE LYMPHBLASTIC LEUKEMIA
Musa Karakukcu¹, Ekrem UNAL¹, Fatma Turkan MUTLU¹, Mehmet Akif OZDEMIR¹, Turkan PATIROGLU¹, Yusuf OZKUL²
¹Erciyes University, Faculty of Medicine, Department of Pediatrics, Kayseri, Turkey, ²Erciyes University, Faculty of Medicine, Department of Genetics, Kayseri, Turkey

Introduction: Treatment associated morbidity has emerged as a topic of interest, as the over-all survival of childhood acute lymphoblastic leukemia (ALL) reached to approximately 80% with the current protocols. Methotrexate (MTX), which interrupts folate metabolism, is widely used in the treatment of ALL, but causes some undesirable toxic effects. Methylenetetrahydrofolate reductase (MTHFR), a fundamental enzyme that regulates folate metabolism, serves as a telescope to the pharmacogenetic world. In this study, we aimed to investigate the association of the MTHFR gene C677T polymorphisms with the prognosis and toxicities after medium dose MTX (1000 mg/m²/36h) infusion. Methods: We investigated the relationship between MTX related neutropenia, mucositis, and MTHFR polymorphism. Toxicities were assessed using the Common Terminology Criteria for Adverse Events (CTCAE), Version 4.0. The registration included nadir laboratory values, occurrence of fever (hospitalization with temperature >38°C), and delay in the chemotherapy regimen. Results: 196 courses of MTX infusion at medium dosage from 50 children (29 boys and 21 girls; mean age 6.0±2.8, range 2-12 year) with ALL treated according to the BFM Turkish national ALL protocol at Erciyes University, Children’s Hospital, Kayseri, Turkey, between January 2004 and December 2012, were enrolled to this study. Subjects with wild type (CC), heterozygote, and homozygote MTHFR polymorphism was 26 (52%), 20 (40%), and 4 (8%), respectively. The distribution of wild type (CC), heterozygote (CT), and homozygote (TT) MTHFR polymorphism for the 196 MTX courses was found to be 100 (51.0%), 80 (40.8%), and 16 (8.2%), respectively. We found a correlation between MTHFR polymorphism and neutropenia and mucositis. However, no effect of MTHFR polymorphism on the prognosis and outcome of the patients were determined. Conclusions: Our data suggest that MTHFR gene variants play a critical role in MTX related mucositis and neutropenia in ALL patients. Further studies about the genotyping of folate pathway gene variants might be useful for reduction of chemotherapy toxicity. Furthermore we speculate that genetic fingerprints might improve the survival by individualized dose adjustments of chemotherapeutic agents.
**Concurrent Development of Myeloproliferative Diseases with Clonal B-Cell Lymphocytosis, With an Array CGH Test**

Hyunjung Kim, Soyoung Shin, Myungshin Kim
The Catholic University of Korea Seoul, Korea

**Introduction:** We analyzed the array CGH by isolated B lymphocytes in patients with concurrent development of myeloproliferative disease (MPN) with clonal B-cell lymphocytosis (CBL). Therefore we investigated the patho-mechanism of this disease.

**Methods:** We collected the patients, who had both MPN and CBL from January to December, 2014 in Saint Mary’s hospital. JAK2 V617F mutation tests were performed by a real-time PCR kit from two samples (whole blood and isolated B lymphocytes). The bone marrow karyotype was analyzed and the array CGH was performed using a SurePrint G3 Human CGH Microarray 8 X 60K kit (Agilent Technologies, USA) according to manufacturer’s protocol. **Results:** There were two patients with both MPN and CBL were from 6531 patients who performed bone marrow test. The diagnosis of one patient was essential thrombocytopenia (ET) with chronic lymphocytic leukemia (CLL), the others had both ET and CBL. Both patients revealed normal karyotype. JAK2 V617F mutation were present in whole blood, but not in B lymphocytes in case of patient 1 and were present in both whole blood and B lymphocytes in case of patient 2. The array CGH revealed a gain of 4q28.3 in patient 1, and the gain of 4q28.3 was previously reported in non-Hodgkin’s lymphoma. In case of patient 2, a loss of 22q11.22 and a gain of Xp22.31 in patient 2 were found. Thedelelton 22q11.22 is a common genetic imbalance observed in CLL and a gain of Xp22.31, which may be a kind of benign copy number variation. The B cells of both patients did not have any genetic imbalance, which were reported in myeloproliferative disease, including ET. **Conclusions:** The JAK2 V617F mutation was present in isolated B lymphocytes of patient 2 but not patient 1. An array CGH of the B lymphocytes of the patients revealed a gain of 4q28.3, deletion 22q11.22. The B cells of neither patient had a genetic imbalance specific to myeloproliferative neoplasms (MPN). The results may be suitable to a hypothesis that a different oncogenic mechanism may underlie in each cell lineages in the concurrent development of lymphoid neoplasm and MPN. The array CGH may be helpful in identifying the patho-mechanism in cases of the concurrent development of lymphoid neoplasm and MPN.

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**BCR-ABL GENE MUTATIONS IN IMATINIB RESISTANT CML PATIENTS IN OUR CENTRE**

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**Introduction:** Introduction of tyrosine kinase inhibitors (TKI) dramatically improves the treatment and survival of patients with chronic myelogenous leukemia (CML). TKI resistance remains an important problem in therapy of CML. BCR-ABL gene mutations are among the most significant causes of TKI resistance. **Methods:** We have analyzed molecular response in 47 patients with CML, 22 females and 25 males, treated with Imatinib (IM) as a front or second line treatment. Only 9 patients were treated with Imatinib as a front-line therapy, while 38 patients were previously treated with hydroxyurea or/and interferon. Median duration of CML is 8 years (3 months-16 years). Median duration of IM treatment is 5 years (3 months-9 years). Forty patients (85%) had complete hematological response, 66% had complete cytogenetic response and 55% had major molecular response (MMR). BCR-ABL mutations were analyzed in 16/47 (34%) patients with poor or suboptimal molecular response to Imatinib. Mutations in BCR-ABL gene were investigated by RT-PCR/nested PCR analysis and sequencing, after RNA extraction from peripheral blood monocytes. **Results:** BCR-ABL mutations were detected in 7 of 16 (43%) analyzed patients. Molecular response (MR) was analyzed in 47 patients in at least one up to 6 time points. MMR was achieved in 26/47 (55%) patients, 13/47 (27.5%) patients had MMR at 4,0-4,5 log and 13/47 (27,5%) had MMR at 3,0-4,0 log. BCR-ABL mutations were analyzed in 16 from those 21 patients with poor MR. We detected 6 different mutations in 7 patients: T315I, M244V, G250E, Y253H, E279G and M318V. T315I mutation was detected in 3 patients, M244V in 2 and all other mutations were detected in one patient. Three patients had double mutations: one patient had M244V & G250E mutations; one had M351T & Y253H; and one had T315I & E279G. Three of these 7 patients (one with T315I, one with M244V, one with double mutation M244V & G250E) died due to disease progression. Four are still alive, but only one patient is in hematological and clinical remission despite the coexistence of two mutations (T351I & E279G). **Conclusions:** Our results have shown high frequency of BCR-ABL mutation, probably due to the fact that almost 80% of patients (39/47) were heavily pretreated with HU or/and interferon and long duration of CML disease before IM treatment. Unfortunately, other TKI inhibitors (nilotinib or dasatinib) are not available in our country and we could not make adequate change in the treatment of patients with BCR-ABL mutations.

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**THE ROLE OF THE INTERNET AND MOBILE DEVICES IN CONTINUING PROFESSIONAL DEVELOPMENT FOR MORPHOLOGY**

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**Introduction:** The past decade has witnessed significant developments in digital morphology and its use for continuing professional development (CPD) for healthcare scientists. The team over this time have generated image galleries, depicting multiple examples of variations in morphology, along with malaria images from different species. Initial CPD materials were provided on web pages as text and images, subsequently quizzes were created to supplement the web site training and provide evidence for CPD. Following on from this a parallel project working on point of care testing started developing CBC Apps, the project described here aims to indicate the processes undertaken and how this could be used for morphology on mobile devices. **Methods:** Images for all CPD materials have been developed using the Zeiss Axio Imager M1 microscope and associated software. Web development was carried out using Adobe Dreamweaver. A specialist App developing team was used to develop the “What is a full Blood count App”, which will be used as the basis for future Apps. **Results:** Across the four products over 300 individuals have accessed the sites for the purposes of training and CPD. Pilot access to the peripheral blood and malaria galleries have included 42 international participants. Following expert review the quizzes went live in January 2014. Malaria ETC has been trialled by 200 laboratory professionals. The ‘What is a full Blood count’ App developed as part of a point of care testing project, created in App environment to provide galleries to allow image comparison, to aid diagnosis. The ICSH morphology nomenclature project has been completed and has been associated with images used within the CPD galleries mentioned. **Conclusions:** With the development of the ICSH morphology nomenclature project the group provided images to support the standard definitions that have been approved (www.morphology.mmu.ac.uk). The group plan is to propose to the ICSH to develop these further and provide a mobile device friendly version or App, and this will be presented as a formal proposal at this meeting.
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PLASMA CELL MYELOMA WITH MULTIPLE CRYSTALINE DEPOSITION IN BONE MARROW AND KIDNEY: A RARE CASE IN KOREA
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Introduction: The crystalline deposition of plasma cell or histiocytes can be seen in plasma cell myeloma. However, crystalline inclusion within the cytoplasm of tubular epithelial cells or podocytes in kidney is an uncommon phenomenon, which are known to be associated with Fanconi syndrome. The accumulation of crystalline in tubular epithelial cells or podocytes is considered as a consequence due to resistance of variable domains of monoclonal kappa light chain to degradation by proteases in lysosomes. Herein, we report a rare case of plasma cell myeloma with multiple crystalline inclusion in plasma cells and histiocytes of bone marrow, and also tubular epithelial cells and podocytes in kidney of a patient with the only representative symptom as proteinuria. Methods: A 52-year-old woman was referred for work-up of increased creatinine and proteinuria. Monoclonal gammopathy with IgG and kappa type was observed in both serum and urine immunofixation. Except for the free kappa/lambda light chain ratio (24.58), other clinical laboratory results including quantitative value of IgG showed within reference ranges. In the bone marrow aspirate, plasma cells and histiocytes with needle shaped crystalline inclusions were occasionally observed (more than 10% of absolute nuclear cells). The case was diagnosed with plasma cell myeloma. However, to find the existence of crystalline deposits in kidney, renal biopsy was done. Results: In the renal biopsy, crystalline structures in cytoplasm of some tubular epithelial cells and segmented glomerulosclerosis were observed by polarizing microscopy. And electron deposits with some crystalline structures in the cytoplasm of podocytes and tubular epithelial cells were found from electron microscopy. Formalin-fixed sections were positive for light chains using immunohistochemical stain. Congo-red stain showed negative result. Although proximal tubular epithelial crystalline was found, this case did not show any clinical laboratory evidences with Fanconi syndrome, such as hypokalemia, hypophosphatemia, and metabolic acidosis, except for proteinuria and weak glucosuria. Conclusions: This case highlights very rare case showing crystalline deposition of plasma cells and histiocytes of bone marrow and tubular cells and podocytes of kidney. Based on this histopathologic findings, there can be a patient presenting only proteinuria as a symptom due to focal deposition of crystalline inclusions. Therefore, intensive further work up is needed in multiple myeloma patients with proteinuria.

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EVALUATION OF CELLAVISION DM96™ IN MALIGNANT HEMATOLOGICAL DISORDERS
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Introduction: The bone marrow (bm) aspirate smear examination is a critical parameter in the diagnosis and management of malignant hematological disorders. Cella vision dm96™ is an automated image analysis system that presents images of blood cells and suggests cell classification for white blood cells. In this study, we evaluated the dm96™ in the enumeration of bm aspirate smear and the clinical performance of dm96™ in the diagnosis and management of malignant hematological disorders. Material and method: bm aspirate smears were collected from 62 patients with malignant hematological disorders from various types with the diagnosis of normal bm (16), acute lymphoblastic leukaemia (10), acute myeloid leukaemia (18), b-cell chronic lymphoproliferative disorders (13), and myelodisplastic syndrome (5). Were examined by the dm96™. Slides were prepared with sp-100 sysmex from bm aspirate smears were collected and also counted on the symsc xe-2100. The classification of these 62 patients was made according to the their flow cytomtery results. Statistical analysis was performed using microsoft_excel software. For correlation analysis, we used to evaluate differences between the percentage of blast cells detected by dm96™ manual microscopic analysis were performed by clinical hematologist. The reclassified results from the dm96™ for all cell classes were compared with manual microscopic / flow cytometric results. Results: The correlation coefficients between results from dm96™ after reclassification of cells and the results obtained by manual microscopic analysis were 0.99 (Blast cells), 0.70 (Band neutrophils), 0.90 (Segmented neutrophils), 0.90 (Lymphocytes), 0.77 (Monocytes), 0.74 (Myelocytes), 0.70 (Metamyelocyte), 0.65 (Promyelocyte) and 0.51 (Eosinophil). The lowest correlations were observed for eosinophils and promyelocytes. Correlation coefficient between the percentage of blast cells detected by dm96™ and flow cytometry analysis was 0.96. Conclusion: in this study, we demonstrated that the results obtained from dm96™ correlated well to those obtained by manual microscopic analysis and flow cytometric analysis of all bone marrow smears with different haematological disorders. Although the dm 96™ is designed for the peripheral blood differential count, we suggest its use in bone marrow aspirate smears, and also the system is very useful tool for correct and rapid hematological evaluation in diagnosis of malignant hematological disorders.
513 DEVELOPMENT OF A PILOT HAEMATOLOGY VIRTUAL SLIDE LIBRARY AS A TOOL FOR MORPHOLOGY EDUCATION
Poomahal Kumar1, Sarah Halawani2, Fifin Intan3, Szu Hee Lee4
1Haematology Dept, Pathology North, Royal North Shore Hospital, 2Royal College of Pathologists of Australasia, 3RCPA Quality Assurance Programs, 4Haematology Department, St George Hospital, SEALS Central Sydney Australia

Introduction: Virtual slides have certain advantages over glass slides for Haematology morphology education as slides can be readily distributed, identical images are viewed by all users and images can be accessed remotely via the Internet. The Royal College of Pathologists of Australasia (RCPA) Haematology eCases Library is a pilot web-based virtual slide library that has been developed by the Sydney-based New South Wales/Australian Capital Territory (NSW/ACT) teaching program for medical postgraduate trainees in Haematology. Methods: RCPA tutors were invited to contribute slides of blood smears, bone marrow aspirates (BMA) and trephine biopsies (BMTB) of haematological disorders to the Library. Glass slides were scanned with an Aperio Scanscope OS. Blood smears and BMA were scanned at X100 magnification under oil immersion, while BMTB were scanned at X40 magnification. Virtual slides were displayed using Aperio Webscope, a web based platform-independent slide viewer. The Library is searchable by topic, sub-topic, specimen type, contributor or text. The sub topic search categories for neoplastic disorders correspond to the WHO Classification of Tumours of Haemopoietic and Lymphoid Tissues, 2008. For each case, users are presented with the blood count, brief clinical details and virtual slides. Users may then access further information including descriptions of the morphology, investigations, additional images, diagnosis, comments and references. User surveys were conducted in 2013 and 2014. Results: Starting in 2013, the Library has accrued 141 cases comprising 104 blood smears, 30 BMA and 21 BMTB slides. In 2014, there were a total of 1772 case views by trainees and Fellows of the RCPA. Among these, 1463 were returning users and 309 were new users. In user surveys, trainees who accessed the Library agreed that it was a useful tool for morphology education and indicated that their usage of the Library would increase if more cases were added Conclusions: A pilot web-based Haematology virtual slide library has been developed as an educational tool to enable trainees to improve their skills in the interpretation of blood and bone marrow morphology. Further contributions of cases by tutors are expected to increase the size of the library in the future.

514 SEMI-AUTOMATED SEGMENTATION AND MEASUREMENT OF CYTOPLASMIC VACUOLES IN NEUTROPHILS WITH GENERAL-PURPOSE IMAGE ANALYSIS SOFTWARE
Nobuo Masauzi1, Maki Mizukami2, Misaki Yamada2, Sayaka Fukui2, Nao Fujimoto2, Kie Horioka1, Shigeru Yoshida1, Sanae Kaga1, Keiko Obata1, Shigeji Jin1, Keiko Miwa1, Taisen Mikami1
1Department of Medical Laboratory Science, Faculty of Health Sciences, Hokkaido University Sapporo, Japan, 2Graduate School of Medical Sciences, Kanazawa University Kanazawa, Japan,
3Department of Clinical Laboratory, Japanese Red Cross Medical Center Tokyo, Japan, 4Division of Medical Technology, Hokkaido Industrial Health Management Fund Sapporo, Japan, 5Graduate School of Health Sciences, Hokkaido University Sapporo, Japan, 6Department of Cardiovascular Medicine, Osaka University Graduate School of Medicine Osaka, Japan

Introduction: For computer-aided image analysis of blood cells, the segmentation of the nucleus from the cytoplasm or lymphoblastic cells from a peripheral blood film had been reported. However, we cannot find any report concerning the characteristics of small structures in the cytoplasm. We developed a new image analysis method using Photoshop(r) and Image-J, called PS-IJ. The method semi-automatically segmented vacuoles from the cytoplasm, and measured the area of every vacuole. Methods: For formation of cytoplasmic vacuoles in neutrophils, we stored whole blood samples from five healthy volunteers at 4 degree Celsius with three different anti-coagulant agents (ACAs). These were EDTA-2K, heparin, and Sodium citrate. These blood sample tubes were stored in two different preserving manners (PMs). For the first manner (P1), the only one blood sample was preserved for six different times of observation. For the second manner (P2), six tubes were stored for the same times of observation. The enlargement of the entire cell area (ESA) and increase of vacuole formation (VF) were observed in neutrophil after preservation. Using PS-IJ, the segmentations were conducted and the area of vacuoles were measured by counting pixels inside of the vacuoles. The pixels inside of the entire neutrophil were counted using PS-IJ for measuring ECA. The ratio of all the vacuoles portions to ECA (V/EGA ratio) was calculated for the comparison of the differences in VF among three ACAs and between two PMs. For evaluating the accuracy in segmentations of vacuoles with PS-IJ, the V/EGA ratios calculated with results by PS-IJ were compared with those by human eye and IJ (HE-IJ). In all statistical test, p<0.05 was considered significant. Results: The enlargement of the ECA and increase of VF seemed to differ among the three ACAs and between the two PMs from HE observation. The evaluation with PS-IJ indicated that these increases were significant, and the starting time of the increase in VF significantly differed among the three ACAs. The maximum values in both the ECA and V/EGA ratio were also revealed to be significantly different between the two PMs. The V/EGA ratios with PS-IJ demonstrate a very good correlation (r=0.90, p<0.01) to those with HE-IJ, suggesting that PS-IJ enables correct determination of the vacuole portions in every cell compared to that with HE-IJ. Conclusions: Our PS-IJ method can successfully segmented vacuoles in the cytoplasm and will become a useful tool for full-automated differentiation and determination of blood cells including of malignant blast.
EXTERNAL QUALITY ASSESSMENT SCHEME (EQAS) FOR BLOOD SMEAR INTERPRETATION: EVALUATION OF THE RESULTS AFTER FOUR YEARS EXPERIENCE
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1Hemotherapy-Hemostasis Department. Hospital Clinic. Barcelona, Spain, 2Standardization in Hematology Committee of the SEHH Spain

Introduction: Microscopic examination of peripheral blood (PB) is an important diagnostic tool. The Spanish Haematology and Haemotherapy Society (SEHH) decided to set up a PB smear scheme. We showed the results obtained after 4 years experience (2011–2014). Methods: A total of 24 PB films were stained with MGG and every two months they were sent to 116 participants: 2 Acute Promyelocitic Leukaemia (APL), 4 Hairy Cell leukaemia (HCL), 1 Sickle Cell Anaemia (SC), 2 Mantle Cell Lymphoma (MCL), 1 Follicular (FL), 1 Sézary syndrome (SS), 1 infectious mononucleosis, 6 AML, 1 ALL-B, 1 Primary Myelofibrosis (PMF), 2 Chronic Myeloid Leukemia (CML), 1 Bernard-Soulier syndrome (BS) and 1 Plasmodium falciparum (P). Patient details were included and the participants were asked to select: Up to 4 significant morphology features, the diagnosis and the immunophenotypic or cytogenetic abnormalities suggested. They received a report with: 1) Morphological alterations identified by the participant and the referent ones 2) Frequency of the morphological alterations and diagnoses reported, 3) Clinical background of the patient including relevant immunophenotypic or cytogenetic data and the diagnosis, 4) Abnormal cell images and 5) Discussion. Results: The correct diagnosis for the APL surveys was reported by a mean of 87% and 97% of the participants. For the HCL surveys, hairy cells were reported by a mean of 81%, 83%, 94% and 86% of the participants. A total of 94% participants informed sickle cells in the SC survey. Reactive lymphoid cells were reported by the 99% of the participants in the smears corresponding to mononucleosis. The percentages in the morphological diagnostic were not so good for the MCL, SS an FL surveys: 51 %, 34 %, 17 % and 45% confirming that neoplastic lymphoid cells are the most difficult cells to classify by using morphologic features only. Acute leukemia surveys had very good morphological diagnosis percentages, (99/97/96/93/90/97%). Nevertheless 24% of the participants reported atypical promyelocytes in a LMA with nucleophosmin mutation. With respect to the PMF and CML, a mean of 68, 87 and 94% of the participants suggested the correct diagnosis in PMF and CML smears respectively. Abnormal platelets in BS were seen by the 96% of the participants and Plasmodium by the 98%. Conclusions: Continued professional development in blood film morphology by an EQAS is strongly recommended since morphological examination of PB cells is the first analytical step in most of the diagnosis and it is a useful aid for the indication of further necessary tests.
HISTOPATHOLOGICAL STUDY OF PRIMARY MYELOFIBROSIS (PMF) AND ESSENTIAL THROMBOCYTHAEAMIA (ET)  
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Introduction:  Myeloproliferative neoplasms (MPN) form part of spectrum of myeloproliferative neoplasms. Both ET and early prefibrotic phase of PMF (PF-PMF) present with thrombocytosis and show several overlapping/common histological features; however, these two conditions differ greatly with respect to prognosis and rates of transformation to leukemia.  

Aim and objectives  
1) To study the histopathology of ET, PF-PMF and PMF using a scoring system including karyotype analysis  
2) To determine whether the scoring system reliably distinguishes ET and PF-PMF.  
3) To compare the laboratory and histopathological features of PMF and ET with JAK2V617 mutation status.  

Methods:  A total of 134 cases were included after reclassification and applying the exclusion criteria. There were 27 cases of ET, 13 cases of prefibrotic phase of PMF (PF-PMF) and 94 cases of PMF. The histomorphological features of all cases were studied in detail with a score applied to various histomorphological features ranging from 0-2, which included 16 parameters on bone marrow trephine biopsies such as increased cellularity, granulocyto, erythroid and megakaryocyte cellularity, left shift in myeloid lineage, megakaryocytes, type of megakaryocyte nuclei, cluster formation, cluster density, sinusoidal dilatation, intrasinusoidal haematopoiesis and grade of reticulin.  

Results:  
1) The scores obtained were: ET, 5-9 (median 8), PF-PMF, 12-17 (median 15) and PMF, 11-21 (median 17) with low scores associated with marked reticulin fibrosis. 
2) The JAK2V617 positive PMF and WT PMF had significant differences with regards to hemoglobin levels (P value=.04) and clinical parameters such as spleen size (P value=.02). With regard to JAK2V617 positive ET and WT ET significant differences were seen with regard to platelet counts (P value=.02) and large clusters of megakaryocytes (P value=.047). 
3) In addition clonal abnormality was detected in 41% of PMF, 50% in PF-PMF who were tested. All cases of ET showed normal karyotype. 

Statistically significant features in favour of prefibrotic phase of PMF, which were not seen in ET were  

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<tr>
<td>PF-PMF</td>
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<td>ET</td>
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<tr>
<td>Left shift, myeloid lineage</td>
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<tr>
<td>Sinusoidal ectasia (31%, P value=.008)</td>
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Conclusions:  There appeared to be clear differences in the scores associated with ET and PF-PMFusing our scoring system. JAK2 positivity was associated with significantly higher spleen size and haemoglobin (PMF) and platelet count (ET). The only significant histological difference on trephine biopsies was the presence of large clusters of megakaryocytes in JAK2V617 positive ET.
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A NEW APPROACH TO MEASURING PLATELET AGGREGATION AND PLATELET-LEUCOCYTE CONJUGATE FORMATION IN A SMALL VOLUME OF FIXED WHOLE BLOOD
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Introduction: Platelet function testing is of value in determining the relative impact of different platelet agonists and inhibitors on platelet function. Investigations can be limited by the volume of blood that is available. The aim of this study is to assess platelet aggregation (PA) and platelet-leukocyte conjugate (PLC) formation in a small blood volume using a 96-well plate format.

Methods: Platelet function was assessed in whole blood from healthy volunteers using 96-well plates coated with: arachidonic acid (AA, 0.03-1mM), ADP (0.3-30µM), collagen (0.1-10µg/ml) and TRAP (0.1-10µM). To check the suitability of this assay to detect platelet inhibition the effects of aspirin, the P2Y12 inhibitor cangrelor, the GPIIbIIIa blocker MK-852 and the PSGL-1 blocker KPL-1 were investigated. Whole blood (46µl) was added to each well and the plate was shaken for 5mins at 1000 rpm at 37ºC; a fixative solution AGGFix (Platelet Solutions Ltd, Nottingham, UK) was applied to stop platelet stimulation and stabilise samples. Both PA, as a fall in the number of single platelets, and PLC formation were measured in the same fixed sample by flow cytometry without red cell lysis and within 3 days after fixation.

Results: Full dose-response curves to 4 agonists were generated using 1.2ml of whole blood. Aspirin inhibited AA-induced PA, cangrelor induced dose-dependent inhibition of ADP-induced PA and both agents inhibited PLC formation. Collagen- and TRAP-induced PA was also impaired to a different extent by in vitro addition of either antiplatelet agent. PLC formation was readily measured in the same samples as PA and was abolished by the addition of KPL-1, which had no effect on PA. In the presence of MK-852 PA was not completely inhibited while PLC formation was dramatically increased and the latter was only partially inhibited by further addition of KPL-1.

Conclusions: The assessment of PA and PLC formation in one fixed whole blood samples offers a reliable method for obtaining two measures of platelet function using a very small volume of blood.

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VALIDITY OF PLATELET ANALYSIS DETECTED BY BECKMAN COULTER LH780 HEMATOLOGY ANALYZER VIA CELLAVISION DM96 AUTOMATED DIGITAL CELL MORPHOLOGY
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Introduction: The analysis of platelets is normally flagged by hematological analyzer to remind the user for problem solving in abnormal results. We determined an accuracy of platelet flags; platelet counts, platelet clumps and giant platelets generated by Beckman Coulter LH780 analyzer.

Methods: Peripheral blood of 138 patients with one or more of thrombocytosis, thrombocytopenia, platelet clumps and giant platelet flags detected by Beckman Coulter LH780 analyzer were enrolled for platelet analysis. Platelets on blood smears of all patients were analyzed by CellaVision DM96 as platelet count estimation, thrombocyte aggregation and giant thrombocyte. Platelet count estimation was used for thrombocytosis and thrombocytopenia validation whereas detection of thrombocyte aggregation and giant thrombocyte were used for platelet clumps and giant platelets evaluation, respectively.

Results: The findings showed no difference of platelet counts between both analyzers with an R² of 0.877. Bland-Altman plots did not show any systematic bias. The results of giant platelets and platelet clumps flags were 63.77% and 62.32% of agreement analysis, respectively due to a quality of peripheral blood smears and misclassification of CellaVision images.

Conclusions: This study demonstrates an accuracy of platelet flags for quantitative analysis generated by Beckman Coulter LH780 analyzers. However, platelet clumps and giant platelet flags should be considered by the user.
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REAL CLINICAL UTILITY OF THE IMMATURE PLATELET FRACTION IN THE DIAGNOSIS OF CHILDHOOD IMMUNE THROMBOCYTOPENIA.
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Introduction: A rapid assessment of platelet production should distinguish between thrombocytopenia due to increased peripheral platelet destruction from decreased platelet production. This study aimed to validate the immature platelet fraction (IPF) in differentiating thrombocytopenia resulting from peripheral destruction as in immune thrombocytopenia (ITP) and Evan’s syndrome, from thrombocytopenia associated with bone marrow failure and aplastic anemia, and its potential use as a diagnostic marker. Methods: A retrospective review of all IPF tests drawn from patients of the Children’s Hospital of Philadelphia was conducted over the past year (10/2013-11/2014). Complete blood count (CBC) and IPF were performed using the Sysmex XN3000. Data were compiled using excel and analyzed using Prism Graphpad software. Results: Over the 1-year period, 293 IPF tests were collected. Based on ICD9 diagnosis codes, 61 patients were diagnosed with ITP (acute and chronic) and Evan’s syndrome, compared to 232 other diagnoses that included diagnoses such as bone marrow failure, acquired aplastic anemia, malignancy, 22q11, or platelet aggregation defects. Patients identified as having ITP had IPFs that ranged between 1.0 to 50.5, with a median of 11.25 (IQR 6.05 – 18.125). In contrast, patients with BMF had a median IPF of 2.5 (IQR 2.05-4.75). We examined the ability of the test to differentiate between bone marrow failure and ITP/Evans syndrome. Using an ROC curve, we determined that the optimal cutoff value for an abnormal IPF was 3.8%. At this value, the sensitivity and specificity of the IPF in distinguishing ITP from BMF was 90% (95% CI 79.5-96.2) and 75% (CI 34.9-96.8) respectively. While the positive and negative predictive value of the elevated IPF were 0.964 (CI 0.877-0.997) and 0.500 (CI 0.211-0.789). Conclusions: Patients diagnosed as having ITP or Evan’s syndrome were more likely to have a high IPF correlating with increased peripheral destruction, in contrast to those patients diagnosed with acquired aplastic anemia or other bone marrow failure syndromes. Patients suspected of ITP with a low IPF should be carefully evaluated for other potential etiologies of thrombocytopenia as the likelihood is high that they may not have ITP. The IPF is a rapid and inexpensive test that can be integrated as a standard parameter to evaluate the thrombopoietic state of the bone marrow.

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IMMATURE PLATELET FRACTION MONITORING IN IDIOPATHIC THROMBOCYTOPENIC PURPURA
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Introduction: The distinction between decreased production and increased platelets (PLT) destruction and/or sequestration is crucial in clinical diagnosis of thrombocytopenia (TCP). In increased PLT destruction TCP, increased megakaryocytes (MK) number as result of an overproduction is often observed, and can be evaluated by the Immature Platelet Fraction (IPF) parameter. ITP is characterized by acute onset, no obvious illness causes, clinical signs of petechiae and bruising, low PLT count. This study aims to monitor the progression of disease and the PLT production recovery through PLT and IPF as indirect index of MK activity. Methods: A 4-year-old girl, admitted to the Emergency Room for petechiae spread after viral infectious episode and moderate splenomegaly. Laboratory test at the admission: blood count (Sysmex XE-2100), peripheral blood smear, coagulation tests (PT, aPTT), bone marrow aspiration (BM), anti-platelet antibodies (PLT-Ab). An initial diagnosis of acute ITP was established. After an early remission, ITP became a chronic condition and needed a steroid therapy. From then after, the patient’s PLT monitoring was performed by the laboratory parameters PLT and IPF. Results: At admission haematological parameters were within age-related reference values, with the exception of PLT (4x10^12/L) and high IPF percentage. Peripheral blood smear review showed giant PLT; in BM erythroid and myeloid lineages showed normal hematopoiesis, while MK hyperplasia was present with prevalence of immature stages and more basophilic cells. Coagulation parameters were within normal age ranges and PLT-Ab present. Up to a year, the disease monitoring showed the following data (last 5 monitoring results): PLT(x10^12/L): from 3 to 6, 9, 15, 4, 5; inverse IPF trend: from 31.4 to 32.7, 25, 36.8, 35.9 (ref. val.3.55; statistical significance: p < 0.001). Conclusions: In ITP, the test trend matches to the impaired recovery in MK production. This case shows a persisting serious TCP and an increased IPF following the PLT fall thus providing immediate indications of the MK recovery related to the steroid therapy. IPF, reliable and easy to use, allows indirect access to the BM activity, detecting megakaryocytopoiesis trend, in diagnosis and follow-up, avoiding the BM as indicated by the guidelines, mostly in pediatric and/or severe TCP.
EVALUATING RELIABILITY OF LOW PLATELET COUNTS ON MINDRAY BC-6800 AND COMPARISON WITH ICSH REFERENCE METHOD
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Introduction: Hematology analyzers generally provide reliable platelet counts within normal range. However, the results have poor reproducibility at low platelets levels and often unacceptable when platelet counts are 20x10^9/L or lower. It is widely known that reproducibility of platelet counts is linked to technology showing largest variation in analyzers using impedance counting method. For below normal levels, reliability improves when technology moves from impedance to optical scatter of laser by fluorescence tagged cells is used. The method proposed by ICSH in 2001 as reference method for platelet counting, combines flow cytometry & immunology to provide reliable platelet counts even at very low levels. However, it is perceived as impractical and costly for routine work. We rely on platelet count results from hematology analyzers used at our institute. However, ICSH Reference method is also used to reconfirm counts when platelets are < 20x10^9/L. BC-6800 (Mindray, Shenzhen, P.R. China), among several advanced technology features such as 3D WBC analysis using SF Cube, counting Reticulocyte & NRBC; also has two modes for counting platelets i.e. PLT-I (impedance) & PLT-O (fluorescence tagged optical scatter). Also has an in-built algorithm to report the more reliable platelet count from among PLT-I & PLT-O results. Methods: Platelets were counted on BC-6800 in PLT-I and PLT-O modes (n=100) and data compared against results from ICSH reference method (PLT-Ref). Results: Platelet counts ranged from 2~777 x10^9/L (n=100). 65 of these had PLT counts < 150 x10^9/L and 34 (out of 65) had PLT counts < 20x10^9/L. PLT-Ref results correlated well with PLT-I & PLT-O data from BC-6800. Coefficient of determination (R^2) between PLT-O & PLT-Ref was 0.998 while for PLT-I & PLT-Ref method, R^2 was 0.989. In 34 cases (PLT < 20 x10^9/L), R^2 were 0.963 (PLT-O & PLT-Ref) and 0.842 (PLT-I & PLT-Ref). Conclusions: BC-6800’s PLT-O results showed excellent correlation with PLT-Ref method and good reliability even at levels < 20 x10^9/L. Using BC-6800’s PLT-O results routinely provides an option to reduce reliance on PLT-Ref method. We also believe that good reliability of PLT-O could save cost & time (over ICSH method), reduce load on blood component services (by reducing over transfusion) to speed up therapeutic intervention.

IPF MEASUREMENT IN SYSMEX XN PLT-F CHANNEL
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Introduction: Reticulated platelets (RP) are immature platelets released from the bone marrow in a wide group of diseases; it is used as a good indicator of megakaryopoietic activity. These newly produced platelets can be distinguished from mature platelets by their content of RNA by flow cytometry. Immature Platelet Fraction (IPF) can be an appropriate marker in thrombocytopenia in order to differentiate an increased thrombopoiesis (due to increased peripheral platelet destruction) from a decreased thrombopoiesis. Sysmex-XN analyser has introduced the PLT-F channel giving more specificity than other analysers in IPF measure. Furthermore, IPF values and RP values are moderately related even though they are not quantitatively identical. The aim of this study is to know if IPF% can be a favourable parameter in differential diagnosis of thrombocytopenias. Methods: 100 patients were examined: 25 control group, 34 chemotherapy treated patients (CT), 24 chronic liver diseases (CLD), and 17 primary immune thrombocytopenia (ITP). Peripheral blood collected into EDTA-K3 tubes was used as sample; complete blood counts were analysed by Sysmex-XN analyser; PLT counts were done by impedance (PLT-I) and fluorescence (PLT-F) channel. IPF% tests were measured by PLT-F channel. ROC curve analysis was performed to determine diagnostic accuracy for IPF%. Results: Platelet count for control group, CT, CLD and ITP was 254.58±53.8, 60.00±30.6, 78.00±15.9 and 54.29±26.6x10^3/µL, respectively. IPF% levels had a significant increase in comparison with control group (control group: 5.59±3.1; CT: 6.47±4.1; CLD: 7.03±3.1; p< 0.001). A significant inverse correlation between IPF% and platelet count was observed in PLT-I (r² = -0.281; p< 0.01) and PLT-F (r² = -0.267; p< 0.01) channels. IPF% was clearly different in ITP compared to other thrombocytopenias (ITP: 19.57±7.9; CT: 6.47±4.1; CLD: 7.03±3.1; p< 0.001). A significant inverse correlation between IPF% and platelet count was observed in PLT-I (r² = -0.281; p< 0.01) and PLT-F (r² = -0.267; p< 0.01) channels. IPF% was been suggested as a reliable marker for differential diagnosis of disorders with abnormal platelet counts and prediction of platelet recovery.
ACCURACY OF PLATELET COUNT IN NORMAL AND HEMOLYTIC ANEMIA USING MINDRAY 6800 AND 3600 AUTOMATED HEMATOLOGY ANALYZER
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Introduction: The estimation of platelet number is important for the diagnostic and treatment in many disorders. Platelet count by impedance technique may be interfere in severe microcytosis or fragmented red cell in various forms of hemolytic anemia even with the use of hydrodynamic focusing methods. false increase in the platelet count occurs when red cell or white cell fragments, microcytic red cells, immune complexes, bacteria or cell debris are included in the reported platelet count. To improve discrimination of platelets from non-platelet particles, optical light scattering method has been developed to count and sized the platelets by a flow cytometry system. In this study we have evaluated and compared the platelet count under Mindray BC-6800, BC-3600 with ADVIA 2120 in thalassemia patients with high number of microcyte and fragmented red cell.

Methods: 327 blood specimens at Thalassemia Research Center, Mahidol University included normal genotyping, alpha-thalassemia 1 heterozygote, alpha-thalassemia 2 heterozygote, beta-thalassemia heterozygote, Hb E heterozygote, homozygous Hb E, Hb H, Hb H-CS, beta-thalassemia/Hb E both splenectomized and non-splenectomized. Homozygous beta-thalassemia, E A Bart’s disease, homozygous CS and iron deficiency anemia cases were measured platelet count using automated hematology analyzer; Mindray BC-6800, BC-3600 and ADVIA 2120. PLT-O parameter from Mindray BC-6800 used fluorescence together with optical platelet counting. While PLT-I in BC 6800 and platelet count from other 2 machines were used impedance principle. Genotyping and iron status were characterized in all samples. Results: The obtained platelet counts were shown in table 1. In normal and thalassemia heterozygotes have platelet number in normal range. Obtained platelet count from all 3 machines show good correlation. The number of platelet in splenectomized beta-thalassemia/Hb E cases is "false high level" in Mindray PLT-I because of increased number of microcyte and fragmented red cells in Mindray BC-6800 is corrected in PLT-O measurement.

Conclusions: Platelet count from all 3 automated hematlogy analyzer were comparable with good correlation. However, in thalassemia cases with splenectomized, platelet number were increased above normal range. PLT-O should be used instead of PLT-I in Mindray BC-6800 due to high microcytes and RBC fragments. Table 1: show platelet count of each thalassemia genotypes obtained from 3 different automated hematlogy analyzers.

IS MEMBRANE FLUIDITY AN IMPORTANT PARAMETER IN PLATELET FUNCTION?
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Introduction: Patients with chronic myeloproliferative neoplasms (MPN) have an abnormal platelet response. Many of them have thrombotic or hemorrhage complications. Fluidity of membranes is important in altering of cell signaling and increased the incidence of thrombosis. Aim Revealing the role of membrane fluidity for altered platelet function in patients with MPNs. Methods: The study was retrospective and included 71 patients with MPN and 12 healthy volunteers. Platelet function was analyzed by using optical platelet aggregation using the reagents ADP, collagen, ristocetin, epinephrine. We have also performed fluorimetric method for membrane fluidity analyse using DPH TMA.

The expression of activation marker(CD62P, CD63), adhesion marker (CD41, CD42a and CD42b) and aggregation marker (CD61, CD41) was done by flow cytometric method. Results: Platelet aggregation in patients with MPN is significantly lower for all stimuli. Epinephrine response was observed at the lowest values of amplitude and slope followed by those obtained for ADP and collagen and ristocetin: (ADP 30.97 vs 70.25, p< 0.001; Collagen 27.2 vs 70.86, p< 0.001; Epinephrine 10.68 vs 73.33, p< 0.001; Ristocetin 28.76 vs 71.7, p< 0.001). The expression of CD42a/CD42b, CD61/CD41 are lower in MPNs patients than controls but without significant differences. There are no correlation between expression of platelet receptors and platelet aggregation response. Patients with altered response for ristocetin have a higher membrane anisotropy corresponding decrease in membrane’s fluidity (altered response 0.22 vs normal response 0.13, p = 0.03) although the expression of CD42a/CD42b is unmodified. No differences were obtained for other reagents statistical value (0.157 vs. 0.120 ADP, 0.154 vs.0.137 collagen, 0.137 vs.0.139 epinephrine). The expression of CD42a/CD42b, CD61/CD41 are lower in MPNs patients than controls but without significant differences. There are no correlation between expression of platelet receptors and platelet aggregation response.

Conclusions: Fluidity of membrane is an important parameter that can influence level and quality of receptor expression and cell signaling mechanism. Our results show that a low fluidity of platelet membrane is associated with low response of platelet induced by ristocetin. The stability of platelet aggregates is influenced not only of the level of receptor’s expression but also the quality of these receptors and signaling mechanism.
617 PLATELETS AND VASCULAR ENDOTHELIAL CELL ACTIVATION IN CHILDREN WITH CONGENITAL HEART DISEASE
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Introduction: The precise mechanisms of the increased incidence of hemostatic abnormalities in congenital heart disease (CHD) have not been determined. Aim of the study was to evaluate the effect of activation of platelets and vascular endothelial cell on the pathogenesis of hemostatic disorders in CHD. Methods: this work was carried out on 40 child with CHD (20 patient with cyanotic heart disease {CCHD} and 20 patients with a cyanotic heart disease {ACHD}) in addition to 15 healthy child as a control group, aged between 1- 10 years. All children were subjected to full clinical examination, CBC, oxygen saturation, echocardiography, bleeding and coagulation time, PT, PTT, FDPs, plasma soluble P- selectin, E-selectin and platelets factor 4 (PF4). Results: Significant reduction in platelets count in CCHD (mean 253± 47.8 x 10³) as compared to ACHD (237±102.6 x 10³) and control (394 ± 43.2 x 10³). Significant prolongation in PT and PTT in CCHD (15.45± 3.55 and PTT 49 ± 17.9 respectively ) than ACHD (12.9± 2.3 and PTT 33.5 ± 7.4 respectively ) and control (PT 13.17 ± 0.71 and PTT 5.74 ± 1.8 respectively ). No significant difference in FDPs between all studies group. Significant elevation in PF4 (mean 55.0 ± 25.5 ng/ml), P- selectin (mean 128.9 ± 42.44 ng/ml) and E- selectin (mean 9461.5 ± 1701.2 Pg/ml) levels were reported in children with CCHD as compared to ACHD (PF4 21± 7.94 ng/ml, P- selectin 80.1 ± 13.2 ng/ml and E- selectin 7969.6 ± 2127.5Pg/ml) and significant increase in both group when compared to control group PF4 (8.1± 4.7 ng/ml, P- selectin 27.83 ± 9.73 ng/ml and E- selectin 6750.00 ± 3204.00 Pg/ml). Significant negative correlation between oxygen saturation and plasma P-selectin in CCHD (r = -0.865 with p value 0.001) and E- selectin (r =-0.401 with P value 0.166) , as well as PF4 (r = -0.792 with p value 0.001) and hematocrit level (r=-0.938 with P value 0.001) in CCHD. Nine cases out of 20 ACHD (45%) had pulmonary hypertension (PH) secondary to increased pulmonary vascular resistance (PVRi) ≥ 2 (cut off point for PH). Conclusions: Chronic hypoxemia in CCHD leads to secondary erythrocytosis and increase viscosity and high shear stress of the vessel wall and platelet surface activation favoring thrombogenesis in the microcirculation. Hemodynamic disturbances with accelerated and turbulent flow may be responsible for increased platelet and vascular endothelial cell activation in ACHD and increase risk for thromboembolic complication.

618 PRECISION, ACCURACY AND REFERENCE VALUES OF IMMATURE PLATELET FRACTION OF INDONESIAN ADULTS USING SYSMEX XE-5000
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Introduction: It is important to know the cause of thrombocytopenia as it is closely related to the management plan. An immature platelet (reticulated platelet) is a young thrombocyte which is closely related to thrombopoietic activity. Sysmex IPF is able to enumerate the count of immature platelet. The aim of this study to evaluate the precision, accuracy, sample stability and its normal range for Indonesian adults. Methods: Calibration and daily quality control were performed on Sysmex XE-5000. This study involved 256 healthy subjects (128 males, 128 females). Accuracy and precision tests were conducted within run and between days using e-CHECK and K3EDTA blood at low, normal and high value. Stability tests were also performed using K3EDTA blood at 3 levels of IPF (low, normal, high) and conducted every 30 minutes for the first 4 hours, then every 2 hours for 24 hours. Results: All precision and accuracy test on XE-5000 were shown excellent results as described on Table 1 and Table 2.

Table 1. Within run precision and accuracy test using e-Check and K3EDTA samples.

<table>
<thead>
<tr>
<th>Sample</th>
<th>e-Check 1</th>
<th>e-Check 2</th>
<th>e-Check 3</th>
<th>Sample</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>21.25</td>
<td>21.63</td>
<td>21.9 (%)</td>
<td>Mean</td>
<td>20.94</td>
</tr>
<tr>
<td>SD</td>
<td>0.78</td>
<td>1.23</td>
<td>1.20 (%)</td>
<td>SD</td>
<td>1.88</td>
</tr>
<tr>
<td>CV (%)</td>
<td>3.68</td>
<td>5.68</td>
<td>2.20 (%)</td>
<td>CV (%)</td>
<td>6.01</td>
</tr>
<tr>
<td>d(%)</td>
<td>(-7.31) - 2.74</td>
<td>(-5.5) – 6.42</td>
<td>(-4.31) – 11.48</td>
<td>d(%)</td>
<td>8.99</td>
</tr>
</tbody>
</table>

Table 2. Between days precision and accuracy test using e-Check.

<table>
<thead>
<tr>
<th>Sample</th>
<th>e-Check 1</th>
<th>e-Check 2</th>
<th>e-Check 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>21.84</td>
<td>21.60</td>
<td>21.68</td>
</tr>
<tr>
<td>SD</td>
<td>0.93</td>
<td>0.89</td>
<td>1.12</td>
</tr>
<tr>
<td>CV (%)</td>
<td>4.28</td>
<td>4.12</td>
<td>5.19</td>
</tr>
<tr>
<td>d(%)</td>
<td>(-6.39) – 5.94</td>
<td>(-6.88) – 7.80</td>
<td>(-4.30) – 12.44</td>
</tr>
</tbody>
</table>

IPF results of K3EDTA blood samples for all level of IPF were remained stable for 2 hours. The reference value was 0.64% - 3.20% and no significant difference between male and female group. Conclusions: The precision and accuracy of IPF on Sysmex XE-5000 was showed good results (CV < 10 for all criteria). The sample stability for IPF analysis using K3EDTA blood was 2 hours at room temperature. Reference value for Indonesian adults was 0.64-3.20%.
716 INCREASED RESISTANCE TO OSMOTIC LYSIS INDUCED BY HIGH POTASSIUM ISOTONIC SOLUTION IN SICKLED ERYTHROCYTES.
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Introduction: The osmotic fragility of red cells in hypotonic solutions is well understood while there are few or no information on the responses to isotonic solutions with reversed sodium and potassium ion contents. We have studied the effects of coconut water on the osmotic fragility (OFT) of sickled red cells (HBS) and erythrocytes from hemoglobin genotypes (AA, AS) as controls with a view to highlight their responses to hypotonic solutions after exposure to an isotonic solution with very high potassium ion. Methods: A total of 30 blood samples comprising of HBSS (10), HBAS (10) and HBAA (10) were collected for the osmotic fragility experiments before and after treatments with equal volume of a high potassium medium (Cocos nucifera water). We recorded the absorbance of the mixture with a standard spectrophotometer at 540nm. The average values recorded were plotted against the different concentration of sodium chloride ((NaCl) used. Results: All the samples treated with cocos nucifera water have significantly reduced osmotic fragility (P< 0.05, respectively) irrespective of their haemoglobin genotypes when compared with their lytic values before treatment. Conclusions: We conclude that blood samples treated with high potassium medium resists osmotic lysis as much as possible. We hypothesize therefore that cocos nucifera water and possibly other high potassium isotonic media may reduce the tendency of sickle red cells to osmotic pressure lysis and possibly prolong the red cell’s life span. A possible therapeutic measure is suggested especially in a resource poor setting.

717 RDW-SD AS A TOOL FOR THE DIFFERENTIAL DIAGNOSIS OF MACROCYTOSIS
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Introduction: Macrocytosis is defined as a mean corpuscular volume (MCV) >100FL. Blood cells morphology and biochemical parameters are essential to differentiate megaloblastic anemia (MA) from other causes of macrocytosis. The red cell distribution width (RDW) evaluates the degree of anisocytosis. It can be expressed as a coefficient variation of the MCV (RDW-CV-%) or obtained directly from the width at the 20% height level on the red blood cell histogram (RDW-SD-IL). There are many conditions in which RDW are informative and, according to some studies, when macrocytosis is present, RDW-SD is more sensitive in detecting anisocytosis. Methods: We analyzed 152 samples with macrocytosis (78 with anemia; 74 without; 26 suggestive of MA). They were divided in 8 groups: on chemotherapy, hydroxyurea, alcoholic/hepatic disorders, hemolytic anemia, aplastic anemia, myelodisplastic syndrome, hypothyroidism, and other disorders. The samples were processed in HORIBA, Abbott and Sysmex instruments, in order to monitor the reproducibility and the ability to screen out of range values. The reference values were calculated for each equipment (150 blood donors). Data were analyzed with descriptive statistics, t-student, ANOVA, ROC (GraphPad Prism5). Results: The objectives of this study were to compare the sensitivity of RDW-CV and RDW-SD to detect anisocytosis, in the presence of macrocytosis, and their impact in the differential diagnostic of MA. Reference values span from the minimum to maximum obtained values: RDW-CV 12.03-14.49%; RDW-SD 36.5-46.68fL. Hemoglobin(Hb) values were reproducible (p=0.8727), but MCV, RDW-CV and RDW-SD differ significantly (p< 0.0001). Patients with anemia: Hb median 10.2g/dL[5.4;11.7]; MCV105±7fL, RDW-CV13.3±1.2% and RDW-SD50.2±5.1fL. It was found more cases of MA in “other disorders” group (n=10/63), followed by “hydroxyurea” (n=7/20), and “chemotherapy” (n=4/28). MA and non-MA parameters means showed no significant differences (p>0.05). However, RDW-CV were borderline (MA 14.9±2; non-MA 15.8±3.1) and RDW-SD were elevated (57.3±11.1; 60±11.8, respectively). Regarding to ROC analysis, it was found a better anisocytosis discrimination for RDW-SD, since it presented a higher area under the curve (AUC) than RDW-CV (0.97 and 0.79, respectively). The best cut off for RDW-CV was 14.45% (sensitivity-84.8%; specificity-82.69%) and for RDW-SD was 44.35fL, with improved results for sensitivity and specificity (93.75% and 94.23%, respectively). Conclusions: In macrocytosis, RDW-SD is more sensitive than RDW-CV, improving anisocytosis discrimination. For this reason, RDW-SD may become helpful when MA is considered, as it triggers, earlier than RDW-CV, an alert that requires further actions, like blood smear review and vitamin B12/folic acid quantification.
ALLOIMMUNISATION IN TRANSFUSED SICKLE CELL PATIENTS AFTER RBC ANTIGEN MATCHING

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Introduction: Transfusion is vital in the management of patients with sickle cell disease (SCD). SCD patients over a lifetime will continue to receive RBC transfusions due to ongoing organ damage. Alloantibody formation against RBC antigens is a common complication of transfusion therapy. Transfusion of RBC phenotype matched blood is found to reduce alloimmunization rate. Although in the USA there is no standard of practice, two main recommendations exist; antigen matching only for C, E and K antigens and an extended matching including Duffy and Kidd. However, the impact of these recommendations on alloimmunization is not fully answered. This study therefore aimed at determining the rate of alloimmunization among sickle cell patients before and after transfusion of antigen match blood. Methods: A retrospective review of clinical and transfusion records of SCD patients who received at least two red blood cell transfusions from 1992 to 2013 was performed. These patients had received non-antigen matched and/or antigen matched units. Transfusion units if patients had known antigen matching only for C, E and K antigens if patient had no previous antibodies. If patients had known antibodies the units were matched for C, E, K, Fy, Jk and Jk. Results: Total of 105 SCD patients (79 SS, 11 SC, 8Sβthal and 7Sβthal), 55 male and 50 females were enrolled. 71 and 34 patients received non-antigen matched and antigen matched units respectively. Patients who received non-matched blood comprised of those who received solely non-matched blood and those who received both non-matched blood and matched blood. In the latter, only transfusion histories and antibodies up to the time of switch to antigen matching were included in the analysis. Mean age was 17.1±8.0. There were 54 adults with the remaining 51 aged 1-17 years. Patients received 2711 units in 1624 transfusion episodes. In 31 patients, (25 non-antigen match group and 6 antigen matched group), a total of 59 alloantibodies were detected. Of these, 51 had clear antibody specificities whilst the remaining were occasional antibodies. Percentage alloimmunisation in the patients who received antigen matched blood and patients who received non-antigen matched blood were 17.6% and 35.2%. Following antigen matching, alloantibodies and mean antibody per patient showed about 50% reduction even though mean RBC load was higher in patients receiving antigen matched blood. Immunization risk per RBC units transfused also decreased by a factor of 3. Conclusions: Phenotypic matching of RBC in sickle cell patients reduced alloimmunization by about 50 percent.

<table>
<thead>
<tr>
<th>Patients (n)</th>
<th>Non-matched RBC</th>
<th>Matched RBC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total RBC's</td>
<td>1355</td>
<td>1375</td>
</tr>
<tr>
<td>Mean RBC load</td>
<td>22.6</td>
<td>24.6</td>
</tr>
<tr>
<td>Transfusions episodes</td>
<td>874</td>
<td>756</td>
</tr>
<tr>
<td>No. per episode</td>
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<td>3.6</td>
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<td>Non-matched patients (%)</td>
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<td>Mean Abs per patient</td>
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<td>Mean Abs per Immunized patient</td>
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ABH SECRETOR STATUS AND HIV INFECTION AMONG FEMALE SEX WORKERS IN NAIROBI, KENYA

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Introduction: The secretor phenotype is conferred by the Fucosyltransferase 2 (FUT2) gene and based on expression of the FUT2 gene, approximately 80% of the population are 'secretors' and 20% are 'non-secretors'. In secretors, the products of the FUT2 gene, result in the phenotypic expression of the ABO antigens, on epithelial cells located on mucosal surfaces and in bodily secretions. This raises a number of questions on what role these antigens play at the level of interactions with pathogenic organisms on mucosal surfaces, which are often the first portal of entry for microorganisms including HIV. Methods: This study enlisted 280 female sex workers aged 18-65 years from the Pumwani Majengo sex worker cohort, Kenya. Blood typing was determined by serological techniques using monoclonal antibodies to the ABO blood group antigens. Secretor phenotyping was determined using anti-H specific lectins specific to vaginal and cervical blood group H antigen using the agglutination inhibition technique and correlated to individual HIV sero-status. Results: 92 (32.9%) of the study cases were HIV-1 infected and 188 (67.1%) HIV-1 uninfected. Based on blood group H antigen screening in vaginal and cervical samples, 212 (75.7%) study cases were secretors and 68 (24.3%) non-secretors. The proportion of secretors was significantly higher among women with HIV infection (77/92 = 83.7%) in comparison to HIV un-infected women (135/188 = 71.8%) p=0.029. Based on ABO phenotype stratification, the incidence of HIV infection was higher among blood group A secretors (26/52 = 50%), in comparison to B (12/39 = 33.3%; p = 0.066), AB (3/9 = 33.3%; p=0.355), and O secretors (36/112 = 32.1%; p = 0.028). Conclusions: These findings suggest the non-secretor phenotype may confer a certain degree of protection against HIV infection.

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NINE NOVEL HBA2 MUTATIONS AND SIGNIFICANCE IN BETA-THALASSEMIA DIAGNOSTICS.

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Introduction: Delta-globin gene mutations, either structurally stable and visible or undetectable because of a thalassemic effect or instability, are clinically asymptomatic but may compromise the diagnosis of beta-thalassemia minor. The aim of this study was to report novel mutations in the delta-globin gene found during routine diagnostics and their effect on the expression of HbA2. Methods: Basic diagnostics included determination of the hematological parameters, and separation and estimation of Hb fractions on HPLC and CE. Nine cases were selected for this study according to low HbA2 level or presence of HbA2 variant peaks. Molecular analysis of the delta globin gene was performed by DNA sequencing. Results: Nine novel mutations in the delta-globin gene were found during this study. These included five thalassemic/unstable variants, with low HbA2 level, and four stable variants with visible double HbA2 fractions. Two of the patients were also carrier of a beta-thalassemia mutation and one patient was carrier of a delta-globin gene deletion. Conclusions: HbA2 mutations are either structurally stable and visible on HPLC or CE or unstable or thalassemic, undetectable by basic diagnostics. Alpha-globin gene mutations cause a second HbA2 fraction, which is approximately 25% of the normal HbA2 but a second major fraction of 15-25% will be present besides the normal HbA. A structural delta-globin chain variant will present as a double peak next to the normal HbA2 peak about half the expected amount on HPLC and/or CE. Expression defects of the delta-globin gene reduce the amount of HbA2 by half in normal individuals. Carriers of beta-thalassemia could be missed or be mistaken for iron deficiency or alpha-thalassemia carriers due to a decrease of the otherwise elevated HbA2 level. Therefore, it is important to consider delta-globin gene variants during routine hemoglobinopathy diagnostics.
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**IN VIVO EFFECT OF CARBAMYLATED HEMOGLOBIN ON HBA1C MEASUREMENT BY CAPILLARY ELECTROPHORESIS**

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**HOSPITAL CLINIC BARCELONA, Spain**

**Introduction:** HbA1c is widely used as the gold standard marker to assess glycemic control. Carbamylated hemoglobin (cHb) is a well described interference found in patients with chronic renal failure (CRF) interfering with HbA1c measurement due to its incomplete separation with HbA1c with some ion-exchange HPLC techniques. The aim of our work was to assess potential interferences of *in vivo* cHb on HbA1c, quantification by capillary electrophoresis (CAPILLARYS 2 Flex Piercing, Sebia) in CRF patients. **Methods:** The effect of *in vitro* cHb on HbA1c measurement by CAPILLARYS 2 Flex Piercing was assayed by incubating red blood cells with Potassium Cyanate. The effect of *in vivo* cHb was assayed on samples from patients with CRF (diabetic and non-diabetic) by correlation studies with an HPLC method (G8, Tosoh) that has been previously shown not to have interference with *in vivo* cHb **Results:** cHb resulting from *in vitro* incubation with Potassium Cyanate showed no impact on HbA1c measurements using Capillaries 2 Flex Piercing. Moreover, HbA1c quantification by Capillaries 2 Flex piercing was perfectly correlated with the one obtained on HPLC G8 for both CRF and non-CRF patient groups. **Conclusions:** Sebia Capillaries 2 Flex Piercing analyzer provides a clear HbA1c electrophoretogram easy to interpret, with no interference of *in vivo* cHb. It can be considered a suitable system for HbA1c measurement in laboratories. To our knowledge, this is the first study demonstrating the absence of interference from *in-vivo* cHb on HbA1c measurement by Capillaries 2 Flex Piercing.

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**EVALUATION OF RETICULOCYTE HEMOGLOBIN CONTENT IN IRON-DEFICIENCY ANEMIA AND &BETA;-THALASSEMIA MAJOR**

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¹University Hospital Alexandrovska, Central Clinical Laboratory Sofia, Bulgaria, ²University Hospital Alexandrovska, Clinic of Cardiology Sofia, Bulgaria

**Introduction:** Hemoglobin content of reticulocytes (CHr) provides an appraisal of the bone marrow activity, reflecting the balance between iron and erythropoiesis. In contrast, biochemical markers measure iron supply for the bone marrow and are only indirect indicators of the balance between iron and erythropoiesis. The aim of this study was to evaluate the correlation between CHr and hematological and biochemical parameters of iron status in patients with iron deficiency anemia (IDA) and β-thalassemia major (TM). **Methods:** The study included 15 IDA, 10 TM patients and 40 healthy controls. The hematological parameters were evaluated by ADVIA 2120 analyzer (Siemens, Health Care Diagnostics). **Results:** The mean CHr level was 25.83±3.9 pg in IDA, 22.8±2.1 pg in TM groups (p< 0.005, t-test of Student-Fisher). IDA and TM patients showed a significant decrease of CHr as compared to the controls 33.74±2.1 pg (p< 0.001). The IDA group had significantly lower MCV, MCH, MCHC, reticulocyte number, serum iron (SI) and serum ferritin (SF) and higher soluble transferrin receptors (sTfR) and sTfR/ferritin ratio than those of the TM group (p < 0.001 for all those mentioned parameters). The microcytic and hypochromic cells were more abundant in the IDA group (mean %micro-12.7%, mean %hypo-20%) than in TM group (4.3% and 5.4% respectively, p< 0.001). Significant positive correlations were observed between the CHr levels and Hb, MCV, MCH and MCHC in the both of groups. A negative correlation was found between CHr and serum hepcidin (SH), sTfR levels and sTfR/ferritin ratio and positive correlation between CHr and SF, TSAT and SI in IDA. There was significant positive correlation of CHr with sTfR/ferritin ratio (p< 0.001) in TM group and negative correlations of CHr level with SH, sTfR, SI, SF and transferrin saturation in TM patients. **Conclusions:** In the current study CHr was found to be comparable to the traditional parameters for iron deficiency (SI, SF, sTfR and Hb) and low ferritin concentration for confirming the diagnosis of IDA. In the TM group, lower CHr levels had significant reverse correlation with lower sTfR/ferritin ratio and higher ferritin concentrations due to iron overload. Our data indicate that a panel based on hematologic parameters including CHr may provide an alternative to the traditional hematologic or biochemical panel for the assessment of current iron status.
FRUCTOSAMINE AND PATIENTS WITH THALASSEMA MAJOR

Sotiris Paratiras, Ioanna Fotopoulou, Maria Kaskani, Paraskevi Michail, Eleni Ferentinou, Harris Doliotis, Lampros Bouradas, Artemis Nikolopoulou

Introduction: The measurement of fructosamine in patients with thalassemia and diabetes mellitus type II and its credibility as a glycemic marker in comparison to the measured HbA1c in these patients. Methods: We checked 38 patients (n = 38, 21 male, 17 female) suffering from thalassemia major and diabetes mellitus type II and undergoing treatment. None of patients suffered from hyperthyroidism or hypercholesterolemia and all patients had normal of serum total proteins and albumin. Blood glucose level, hemoglobin A1c and fructosamine were measured as glycemic markers. Measurements of blood glucose were made using the biochemical glucose oxidation method, those of hemoglobin A1c and fetal hemoglobin with the use of HPLC methods and those of fructosamine with the use of chromatography methods. Results: In male and female patients, blood glucose levels were 113.38±25.09mg/dl and 133.94±51.84mg/dl; fetal hemoglobin levels were 9.71%±1.44% and 8.471%±1.352% respectively. A significant positive correlation was found between the two measurements for each patient (r = 0.607, n = 38, p < 0.001). Further tests showed a more significant correlation between the measurements of the two glycemic markers for females (r = 0.667, n = 17, p < 0.003), than males (r = 0.464, n = 21, p < 0.03). Conclusions: In patients with thalassemia and diabetes mellitus a routine measurement of fructosamine every 45 days is required, in order to evaluate the adequacy of the diabetes treatment. This specific test is preferable and corresponds to the HbA1c measurement, especially in patients with thalassemia, as HbA1c (due to the pathophysiology of this hemoglobinopathy and because of the frequent blood transfusions) underestimates the real mean level of glycaemia.

IDENTIFICATION OF HEMOGLOBINOPATHIES IN NON-ENDEMIC COUNTRY- REPORT FROM NORTH ESTONIA MEDICAL CENTRE

Marika Pika, Galina Zemtsovskaja, Kadri Saks, Sirje Leedo, Hanno Roomere

Introduction: Hemoglobinopathy conditions are summarized for a variety of the hemoglobin disorders caused by structural abnormalities and/or alteration in the amount of globin chains. Despite hemoglobinopathies originally found in large regions of Asia, Africa and the Mediterranean Sea area, they are now wide-spread throughout the world because of international migration. The aim of our study was to identify the hemoglobinopathies in Estonia. Methods: In the present study 149 blood samples were received in our laboratory during 04.2011 – 01.2015. All samples were analysed by hemoglobin electrophoresis. In Estonia hemoglobin electrophoresis is performed only in our laboratory. The investigation was done by MiniCap capillary electrophoresis system (Sebia, France). RBC parameters were provided by the Sysmex-XE 5000. The subjects were divided into three groups on the basis of their HbA2 value: group-1 (< 2.5%, n = 42), group-2 (2.5 - 3.5%, n = 74), group-3 (> 3.5%, n = 33). Results: The mean of HbA2 were: group-1 (2.1 ± 0.4%); group-2 (2.6 ± 0.2%); group-3 (5.4 ± 0.4%) and the differences between groups were statistically significant (p < 0.05). Results were interpreted according to the manufactures (Minicap, Sebia, France) recommendations. During the present study, in group-1 were observed 1 HbD variant and 1 a-talassemia (-a/-a); in group-2: 1 HbS/ HbD, 1 HbS, 1 HbE; in group-3 were identified 32 β-talassemia and 1 HbS variant. The following HBB gene mutations were found: c.25_26delAA, p.Lys9Valfs*13 (5); c.112delT, p.Trp38Glyfs (3); c.118C>T, p.Gln40* (1); c.124_127delTTCT, p.Phe42Leufs (1); c.126_129delCTTT, p.Phe42Leufs (1); c.93-21G>A (2); c.316-106C>G (3); c.316-197C>T (1). Conclusions: Despite the fact that Estonia is a non-endemic country with a population of 1.3 million, we found 38 hemoglobinopathy cases. Screening for hemoglobinopathies is very important in non-endemic countries and requires focused attention. A specialized laboratory must provide tests for hemoglobinopathy screening and the results should be confirmed by DNA analysis.
ROUTINE SCREENING FOR BETA-THALASSEMIA IN NORTHEAST THAILAND USING COMBINED HIGH RESOLUTION MELTING (HRM) ANALYSIS AND DIRECT DNA SEQUENCING

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Introduction: β-thalassemia is common among Southeast Asian populations. Rapid screening and identification of β-thalassemia mutations is essential for a prevention and control program. Using a high throughput HRM assay, we have successfully screened for common β-thalassemia mutations in northeast Thailand. Methods: Study was done on 103 Thai subjects with β-thalassemia encountered at our routine screening program. Common β-thalassemia mutations were rapidly identified using HRM assays on four different PCR fragments using an EcoTM real-time PCR machine (Illumina co.-, USA). Those with unknown mutations were subjected to direct DNA sequencing. Results: Among 103 cases examined, the molecular defects found included b37(GAG-AAG; Hb E) (n=44), b41/42(n=23), b7/8(n=20), b24.1G(n=9), b31/32(n=7), b31/32(n=5), b30/31G(n=3), b29(n=2) and b31A-G(n=1). Two other rare mutations identified after DNA sequencing were b30G-A(n=1) and b90CT(n=1). Conclusions: HRM assay is relatively simple and rapid and requires no post-PCR processing and could be applied to both known and unknown β-thalassemia mutations. A combined HRM assay and direct DNA sequencing should prove useful in rapid screening and identification of β-thalassemia in routine setting in the region.

COMPARISON OF RED BLOOD CELL PARAMETERS IN THE DIAGNOSIS OF POLYCYTHEMIA WITH TWO METHODS

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1Hematology Department, Institut Català d’Oncologia, Hospital Germans Trias i Pujol Badalona, Spain, 2Clinical Analysis Department, Laboratori Clinic del Barcelonès Nord i Vallès Oriental Badalona, Spain

Introduction: The erythrocytosis is defined as an increase in the number of circulating RBC of normal size. The increase in erythrocyte mass causes blood hyper-viscosity, difficulty in the intravascular flow and tissue hypoxia. Automated CBC, and particularly some RBC parameters, have a great relevance in the diagnosis of secondary polycythemia and the myeloproliferative neoplasm polycythemia vera, according to the WHO criteria. The aim of this study was to compare hematocrit, RBC and hemoglobin (HB) of patients with polycythemia by two different methods. Methods: Fifty-nine venous blood samples (25 women and 34 men) were taken from patients with increased hemoglobin (cut off 15g/dL in women and 17g/dL in men). Each sample was processed by two different hematologic analyzers, UniCel DXH800 (Beckman Coulter) and Sysmex XE-2100 (Roche Diagnostics). RBC, HB and hematocrit were determined. DXH determines the hematocrit indirectly by impedance (through of volume of RBC, formula Hb 17.6 versus 15.4g/dL, CI:0.17-0.53, hematocrit 48.88 versus 0.85-1.49, p<0.0001), as well as means of HB measurements (17.6 versus 16.4g/dL in women and 17g/dL in men). Each sample was processed by two different hematologic analyzers, UniCel DXH800 (Beckman Coulter) and Sysmex XE-2100 (Roche Diagnostics). RBC, HB and hematocrit were determined. DXH determines the hematocrit indirectly by impedance (through of volume of RBC, formula Hb 17.6 versus 15.4g/dL, CI:0.17-0.53, p<0.0001), as well as means of HB measurements (17.6 versus 16.4g/dL, CI:0.85-1.49, p<0.0001). No differences were observed in the hematocrit determination. Regarding manual versus automatic sampling, (N=24), significant differences were found between means of the three parameters (RBC 5.2 versus 4.58x10^6/L, CI:0.85-2.02, HB 17.6 versus 15.4g/dL, CI:0.17-0.53, hematocrit 48.88 versus 45.45%, CI:0.15-3.22) in XE and DXH analyzers, respectively. As the compared manual samples were processed in a closed tub mode, we hypothesized that pre-analytical factors such as sedimentation or inadequate homogenization could influence the results. Conclusions: Even though the hematocrit is the sole measured parameter with a clear difference in the measurement method, the results were replaceable. RBC measurements had statistical but not clinical relevant differences between methods. HB measurements had statistical and clinical relevant differences between methods. The sampling method can increase the differences between the results, but it needs to be corroborated with more studies. Supported by ISCIII, RTICC, FEDER, (RD12/0036/0044); 2014 SGR225 (GRE), FIJC and Obra Social “la Caixa”
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DIAGNOSIS OF THALASSEMIA AND HEMOGLOBINOPATHIES COMMON IN SOUTHEAST ASIA USING THE V8-CIEF SYSTEM
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Introduction: Capillary Isoelectric Focusing (cIEF) has been continuously developed for identification of hemoglobin (Hb) fractions. We evaluated the V8 cIEF automate Hb analyzer for diagnosis of thalassemia and hemoglobinopathies commonly found among Southeast Asian populations. Methods: Study was done on 691 subjects encountered at our thalassemia diagnostic laboratory at Khon Kaen University, Khon Kaen, Thailand. Hb analysis was performed in all cases using the V8 capillary electrophoresis (Helena Biosciences Europe, Queensway South, UK). Thalassemia genotypes were defined by DNA analysis. Reference range for Hb A2 and diagnostic range for Hb E in heterozygote were also examined. Results: Performance evaluation revealed very good within-run and between-run precision for analysis of Hb A2 and Hb E, with CVs ranging from 0.02-0.09 %. The reference ranges of Hb A2 determined from 51 normal subjects and that of Hb E in 56 Hb E heterozygotes were 2.6-4.0 % and 25.7-33.1 %, respectively. The system identified correctly all cases with β-thalassemia trait and Hb E disorders. Various thalassemia genotypes were observed among 459 adult subjects, 112 carriers of Hb variants and 13 fetal blood specimens. Many Hb variants were noted at the same positions with pre-Hb A, Hb F or Hb S peaks while Hb Constant Spring separates very closely to Hb A2. Hb Bart’s and Hb H were relatively difficult to be reported due to interfering peaks separating at the same region. Conclusions: The V8-cIEF system could accurately identify cases with β-thalassemia heterozygote and Hb E disorders. However as for other HPLC based analyzers, diagnosis of α-thalassemia disease with Hb H and Hb Bart’s might be difficult. For identification of Hb variants, combined analysis with other Hb analyzers is recommended.

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MIE MAP BY ABBOTT CELL-DYN SAPPHIRE: THE SEARCH FOR THE HIDDEN TRAIT
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Introduction: Single-cell optical analysis of red blood cells (RBC) provides information on the cellular hemoglobin concentration and volume of both reticulocytes and mature erythrocytes. Light-scatter measurements and the cell-by-cell characteristics of volume and haemoglobin concentration are represented in the Volume/Hemoglobin Concentration (V/CHC) cytogram or Mie Map; Markers organize the cytogram into nine distinct areas of red blood cell morphology. Graphical data output from automated hematology analyzers, especially those related to red blood cells, have been traditionally ignored in favor of the more frequently used numerical values. We evaluate the reliability of the typical profiles of the cytogram Hemoglobin concentration / Volume (Mie Map), and the percentages of microcytic (MIC%) and hypochromic red cells (HPO%), produced by the CELL-DYN Sapphire analyzer (Abbott Diagnostics, Santa Clara, CA, USA) in the discrimination of IDA and thalassemia trait. Methods: During a 8-week period, all samples with microcytic anemia were analyzed in the reticulocyte mode on CELL-DYN Sapphire analyzer (Abbott Diagnostics, Santa Clara, CA, USA). 400 consecutive patients with microcytic anemia were studied: 220 IDA, 30 ACD, 101 β-thalassemia trait, 30 β-thalassemia trait with concomitant iron deficiency (beta+), 29 α-thalassemia trait. Three professionals, two experts (technician and clinical chemist) and a trainee reviewed the Mie maps, with no information regarding the disease of the patient. The observers made a presumptive diagnosis (genetic or acquired anemia) and the percentages of correct classifications were recorded. Results: On average, 60% of the Mie maps were classified correctly. Conclusion: Mie map patterns from automated analyzers provide clinically useful information that acts as an adjunct to the numerical parameters and at times is even diagnostic of some hematological conditions. Mie map helps in organizing and evaluation of large amounts of data.
PHENOTYPIC EXPRESSION OF HBS A₂, E AND F IN HB E DISORDERS
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Introduction: Hemoglobin E (Hb E) is the most common Hb variant among Southeast Asian population. At the molecular level, Hb E can be classified as a mild beta-plus thalassemia. Interaction of Hb E with beta-thalassemia can lead to Hb E-Beta-thalassemia disease with variable clinical phenotypes. We reported the phenotypic expression of Hbs in heterozygous and homozygous for this common Hb variant. Methods: Data were obtained from 1,123 unrelated subjects with Hb E including 767 heterozygotes and 356 homozygotes. Only those without alpha-thalassemia were recruited. Hematological parameters were collected on standard blood cells counter and Hb analysis was done using capillary electrophoresis which can report Hb A₁ in the presence of Hb E. Results: A remarkable phenotypic variation was noted. In heterozygous Hb E, the levels of Hb A₁, Hb E and Hb F were found to be 3.7±0.4% (1.2-6.2%), 24.6±1.5% (20.6-32.8%) and 0.4±0.9% (0-9.2%), respectively. MCV and MCH values were 78.1±4.1 fl (55.8-98.3 fl) and 25.8±1.6 pg (14.8-30.7 fl), respectively. In Hb E homozygote, the levels of Hb A₁, Hb E and Hb F were 4.5±0.8% (1.8-8.4%), 82.6±6.5% (60.5-96.6%) and 8.8±6.3% (0-31.9%) with reduction in MCV [63.4±5.1 fl (49.0-85.0 fl)] and MCH [21.3±2.1 pg (14.2-35.6 pg)]. Significant differences of Hb E, Hb A₂, MCV and MCH in both heterozygous and homozygous Hb E were observed when the subjects were grouped according to the low and high Hb F. Conclusions: The remarkable variability of the hematological features and the phenotypic expression of Hb A₂, Hb E and Hb F among heterozygous Hb E and homozygous Hb E were observed. It remains to be elucidated whether genetic modifying factors e.g. several SNPs in HBS1L-MYB, BCL11A and EKLF1, known to be associated with Hb F expression in sickle cell anemia and beta-thalassemia, are involved in phenotypic expression of these Hbs in this common hemoglobinopathy.
Introduction: Turnaround time (TAT) is one of the most noticeable signs of laboratory efficiency and is often used as a key indicator of laboratory performance. The prolonged time needed for completion of stat results might cause delays in the treatment of patients. The goal of this study was to evaluate TAT of hemoglobin (HGB) and hematocrit (HCT) in samples of urgent character, which has a established time of release of 30 minutes, and compare TAT before and after processes improvements.

Methods: We used results released data from 2013 and 2014 and the TAT was calculated as the difference between the time when samples were received by laboratory and the time when the Laboratory Information System (LIS) released the results. Results: Between January and May 2013, 1288 results of HGB/HCT were released, as follows: 94.2% released on time (less than 30 min) from what 0.3% in T1 (up to 3 min), 57.2% in T2 (between 3 and 15 min), 39% in T3 (15 to 27 min) and 3.5% in T4 (between 27 and 30 min). In June of 2013 two new systems were implemented, the new LIS and a computerized system for “The automatic update of turnaround”. After that, between June and December 2013, 1372 results were released, as follows: 96.1% released on time, from what 15.2% in T1, 68.3% in T2, 15.2% in T3 and 1.3% in T4. In January 2014, the limit values for automatic release had been updated, and the minimum value of HGB was decreased from 9 to 7g/dl and the HCT from 25 to 21%. Thus, in 2014, 1372 results of HGB/HCT were released, as follows: 97.1% released on time, from what 15.8% in T1, 73.6% in T2, 9.2% in T3 and 1.4% in T4. According to the results during the prescribed period, there was a delay in 5.8% of exams from January to May 2013, 3.9% from June to December 2013 and 2.9% in 2014.

Conclusions: Therefore, we verify that the improvement actions contributed positively in the analytical processes of the HGB/HCT, reducing significantly the release time in most of the results and also the number of delayed results.

Introduction: Approximately 25 percent of the American population is 55 years and older. Reference intervals for laboratory tests are often used to guide clinical decision-making, and for many laboratory tests, these reference intervals are presumed to remain static over the many decades of adult life. Methods: Complete blood counts were compiled from adults aged 55-85 enrolled in seven NHANES cycles from 1999-2012. All samples were measured with either Beckman Coulter MAXM or HmX. Non-Hispanic whites were selected to minimize confounding by race, and stratified by gender. The following exclusion criteria were applied: BMI >35 (females) or >40 (males); recent treatment for anemia; recent hospitalization for heart problems or chest/abdominal surgery; serum creatinine exceeding 221 mmol/L; alcohol consumption >3 drinks/day; positivity for Hepatitis B or C. We constructed reference interval diagrams with 97.5, 95, 90, 50, 5, and 2.5th percentiles plotted against the following age intervals: 55-60 (n=125 Males, 93 Females), 60-65 (144M, 143F), 65-70 (136M, 143F), 70-75 (159M, 131F), 75-80 (132M, 103F), and 80-85 (148M, 175F). Tests studied included red blood cell count (RBC), hemoglobin (HGB), hematocrit (HCT), red cell distribution width (RDW), mean corpuscular hemoglobin (MCH) mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV), platelet count (PLT), mean platelet volume (MPV), white blood cell count (WBC) and 5 part differential. Results: Using data available from the National Health and Nutritional Examination Survey (NHANES) spanning a period from 1999-2012, we examined the effects with aging on reference intervals of commonly measured hematologic tests. Medical exclusions from our base population of non-Hispanic whites aged 55-85 were significant: 1616 out of 2460 males (66%) and 1389 out of 2177 females (64%) were excluded. The remaining subjects (844 males and 788 females) however, were relatively evenly distributed across the various age groups. Age-dependent trends were evident in RBC, HCT, HGB, MCV, PLT, monocyte and lymphocyte count/ percentage, and neutrophil percentage. Those trends which involved red cell measurements tended to be more pronounced in males than in females.

Conclusions: These findings suggest that many common hematologic reference intervals (which often form the basis for clinical decision-making) do not remain static during the aging process, and almost certainly necessitate development of geriatric age-appropriate reference-intervals.
Introduction: Separating plasma from whole blood is the initial step for point-of-care (POC) medical diagnostic tests as is for other tests performed in clinical laboratory. There are various microfluidic methods to separate plasma. Among them, the compact disc (CD) format utilizes centrifugal force to purify acellular plasma supernatant. Because residual cellular components in plasma such as platelets affect the test results, we evaluated a CD format microfluidic system that is adopted for several POC tests currently in use to examine the quality of plasma in the sense of the amount of cellular contaminants and its interfering effect on clinical laboratory tests.

Methods: Samples were collected from ethylenediamine tetraacetic acid (EDTA) and Sodium citrate whole blood specimens. Blood plasma from all specimens was separated using both traditional and CD format centrifuges in order to conduct comparative studies. For EDTA plasma specimens, flow cytometry was used in order to measure residual platelets and spectrometry analysis was conducted for plasma hemoglobin levels in order to determine any additional hemolysis. In addition, enzyme-linked immunosorbent assay (ELISA) was used in order to compare antibody and antigen for hepatitis B. For Sodium citrate plasma specimens, ELISA was used in order to compare soluble P-selectin and Von Willebrand factor (vWF) that are both platelet related biomarkers.

Results: Blood plasma was separated from a total of 56 EDTA whole blood samples using both traditional and CD format centrifuges. Regardless of the separation method, residual platelet counts tended to be higher if the original whole blood sample had high platelet levels. This tendency was more prominent in plasma specimens separated by the CD format method, and the results suggested platelets were being concentrated as residual platelet counts were higher than that of the corresponding whole blood sample. Plasma hemoglobin levels showed no significant difference between the two methods (N=20, p>0.05). And there were positive correlations with regards to hepatitis B antigen and antibody, soluble P-selectin, vWF(r=0.923, 0.466, 0.731, 0.832, respectively). However, 85% of the soluble P-selectin results (17/20) were higher in specimens from the CD format method.

Conclusions: While CD format microfluidic systems can separate blood plasma with little amount of sample in a very short period of time, there is a possibility of interference during the analysis process as well as results in various aspects because of the high number of residual platelets in the plasma specimen.
THE EFFECT OF IN VITRO HEMOLYSIS ON CBC INDICES
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Introduction: Hemolysis is important interfering factor which affect laboratory results especially in complete blood count cell (CBC) data. The previous study reported difference between pre- and post-hemolysis in the hemoglobin (Hb; increase), hematocrit (Hct; increase), mean corpuscular volume (MCV; increase), red cell distribution width (RDW; increase), and platelet (PLT; decrease). This study assessed changes in CBC indices according to the degree of hemolysis. Methods: EDTA-anticoagulated blood samples of 40 healthy adults consisted with 30 males and 10 females were included in this study. The average age was 54 years (range 24-75). CBC was performed on XE-2100 (Sysmex, Japan) and plasma hemoglobin (PHb) was measured with spectrophotometer (Toshiba, Japan). Each sample was divided into two aliquots, and they were placed in 60°C water bath for 30 seconds and 1 minute, respectively. After keeping at room temperature for 3 hours, CBC and PHb measurements were performed again. CBC indices including white blood cell count (WBC), red blood cell count (RBC), Hb, Hct, MCV, mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), PLT, RDW, platelet distribution width (PDW), mean platelet volume (MPV), and plateletcrit (PCT) were analyzed by paired t-test. Differential count was excluded due to functional error by the CBC analyzer after hemolysis. Results: Mean PHb after heating increased from pre-level as 10.6mg/ dL (range 1.6-26.6) to 109.8 mg/dL (range 54.1-173.1) after 30 seconds, and 186.1mg/dL (range 76.9-319.8) after 1 minute. The tubes heated for 30 seconds showed significant differences with pre-heating tubes in all of analyzed CBC indices (p< 0.05). The tubes heated for 1 minute showed differences with pre-heating tubes in all of analyzed CBC indices (p< 0.05), except MCH (p=0.437). Increase of PLT after heating was observed. Conclusions: This study showed that hemolysis critically affects the CBC results, irrespective of the degree of hemolysis. Increased PLT after heating could be due to interference factor, which was discrepant to the previous study. Special attention is required in sampling, transporting and storing the samples for CBC analysis.

SUGGESTION FOR ACCURATE CBC RESULTS TO REMOVE SPURIOUS EFFECTS OF COLD AGGLUTININ ON COMPLETE BLOOD COUNT RESULTS BY DIFFERENT AUTOMATED HEMATOLOGY ANALYZERS: WITH A CASE REPORT OF CHRONIC IDIOPATHIC COLD AGGLUTININ DISEASE
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Introduction: Cold agglutinins aggregate RBCs when exposed to cold and cause spurious CBC results when measured by automated hematologist analyzers. Although cold agglutinins are known to provoke false increase in MCV, effects of cold agglutinin on other parameters of CBC result have not been studied precisely. Herein, we present evaluation results of cold agglutinin effects on CBC parameters by four different automated hematologist analyzers using chronic idiopathic cold agglutinin disease patient’s whole blood sample, and suggest the optimal protocol for accurate CBC results to remove spurious effects of cold agglutinin. Methods: Whole blood sample was collected from a 56-year old male who was diagnosed as idiopathic cold agglutinin disease. 17 separate KEDTA tubes were collected. 4 widely used automated hematologist analyzers were evaluated; XE2100 (Sysmex), XN-1000 (Sysmex), ADVIA2120i (Siemens), and UnicelDxH800 (BeckmanCoulter). The first sample was measured by XE2100, immediately after collection, which served as the reference value. To evaluate effects of storage temperature and duration on CBC parameters, various conditions were applied for a total of 36 measurements (Fig. 1). Comparison of accuracy among each results were analyzed with the unit of delta percentage difference (DPD) from the reference value. Results: The sample stored in 37°C incubator showed least DPD value(0.0–9.3%) among three different temperature conditions. For the protocol of initial storage at 4°C and subsequent incubation at 37°C before the measurement, longer duration of storage increased the DPD value whereas incubation period did not contribute to differences (Table 1). Among four different analyzers, XE2100, XN-1000 and ADVIA2120i were comparable for RBC, Hb, Hct, MCV, MCH, MCHC, and WBC(<15%), whereas platelet count showed larger differences(12.4–39.1%). Importantly, Hb level was not significantly affected by storage temperatures, duration or type of analyzer(0.1–3.2%).

Table 1. Effects of durations for storage at 4°C and incubation at 37°C

<table>
<thead>
<tr>
<th></th>
<th>1 hour at 4°C and 1 hour at 37°C</th>
<th>1 hour at 4°C and 2 hours at 37°C</th>
<th>24 hours at 4°C and 1 hour at 37°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC</td>
<td>1.15</td>
<td>-1.53</td>
<td>-10.69</td>
</tr>
<tr>
<td>Hb</td>
<td>1.09</td>
<td>1.09</td>
<td>1.09</td>
</tr>
<tr>
<td>Hct</td>
<td>0.38</td>
<td>0.76</td>
<td>16.79</td>
</tr>
<tr>
<td>WBC</td>
<td>-4.74</td>
<td>-7.71</td>
<td>-5.73</td>
</tr>
<tr>
<td>Platelet</td>
<td>39.17</td>
<td>42.40</td>
<td>101.84</td>
</tr>
</tbody>
</table>

Conclusions: The optimal protocol for accurate CBC results to remove cold agglutinin effect would be (1) measuring CBC within 1 hour if the sample was stored in 37°C from the collection and (2) measuring CBC after storage in 4°C as short as possible and incubation at 37°C for 1 or 2 hours. XE2100, XN-1000 and ADVIA2120i showed comparable results, whereas UnicelDxH800 revealed greater differences in all parameters except Hb. A Laslike IKZF1, CD-27, N2A/B, PAX5, RB1, ETV6, JAK2, BTG1, CRLF2 and EBF1 over...
eleven months using MLPA (SALSA MLPA P335-B1 kit) in a tertiary care centre in Northern India. The MLPA was done using the genomic DNA as per the manufacturer’s protocol. Electrophoresis and quantification of amplicons was done on Genome Lab GeXP genetic analysis system and the data analysed using Coffalyser software. **Results:** The median age of 104 cases was 7 years (1-67). These included 62 males and 42 females. The gene deletions were seen in **CDKN2A/B** in 37 (35.6%) cases, **PAX5**- 33 (31.7%), **IKZF1**- 30 (28.8%), **ETV6**- 14 (13.5%), **RBL1**- 13 (12.5%), **JAK2**- 11 (10.6%), **BTG1**- 6 (5.8%), and **CRLF2** gene in 4 (3.8%) cases. The **EBF1** gene showed duplication in 3 (2.9%) cases. Overall, the copy number alterations in the above mentioned genes were detected in 67 (64.4%) of the 104 cases. There was defect in only one of the genes in 32 (30.8%) cases, and 35 (33.7%) cases had more than one affected genes. **Conclusions:** Overall, MLPA could detect copy number alterations in at least one of the tested genes in 67 (64.4%) out of 104 cases of B-ALL. More than half of these cases were found to have more than one gene alterations.
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