**O1 MRD ASSESSMENT IN CHRONIC LYMPHOCYTIC LEUKEMIA: WHAT FOR?**

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Minimal Residual Disease (MRD) assessment has become pivotal in the management of a number of hematological malignancies, but only recently made its appearance in the armamentarium utilized for patients affected by Chronic Lymphocytic Leukemia (CLL). This became possible thanks to the use of novel monoclonal antibodies used alone or in combination with standard chemotherapy regimens that allowed to reach a higher number of clinical complete remissions and have opened the path toward the eradication of the disease, at least to a level resulting in a survival benefit. As per international guidelines, MRD-negative status is currently defined as having less than 1 CLL cell in 10,000 leucocytes (0.01% or 10−4). This can be assessed using two distinct approaches, based on Real-Time Quantitative polymerase chain reaction (PCR) or on multicolor flow cytometry. Using either of these methods, it has been shown that MRD status at the end of treatment is one of the most powerful predictors of PFS and OS, independent of the conventional response, patient age and the type or line of treatment. For these reasons, all other less sensitive techniques (e.g. qualitative PCR and 2-colour flow cytometry) should not be used any longer to study MRD status.

**O2 DIAGNOSIS AND TREATMENT OF DIC**

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Disseminated intravascular coagulation (DIC) is a syndrome characterized by systemic intravascular activation of coagulation, leading to widespread deposition of fibrin in the circulation. In patients with DIC a variety of altered coagulation parameters may be detectable, such as thrombocytopoenia, prolonged global coagulation times, reduced levels of coagulation inhibitors, or high levels of fibrin split products. In addition, more sophisticated tests for activation of individual factors or pathways of coagulation may point to specific involvement of these components in the pathogenesis of the disorder. There is not a single test, however, that is sufficiently accurate to establish or reject a diagnosis of DIC. Nevertheless, combination of widely available tests may be helpful in making the diagnosis of DIC and can also be helpful to guide in the selection of DIC patients that require specific, often expensive, interventions in the coagulation system. Recent knowledge on important pathogenetic mechanisms that may lead to DIC has resulted in novel preventive and therapeutic approaches to patients with DIC. Molecular pathways that contribute to inflammatory-induced activation of coagulation have been precisely identified. Pro-inflammatory cytokines and other mediators are capable of activating the coagulation system and downregulating important physiological anticoagulant pathways. Activation of the coagulation system and ensuing thrombin generation is dependent on expression of tissue factor on activated mononuclear cells and endothelial cells and is insufficiently counteracted by tissue factor pathway inhibitor. Simultaneously, endothelial-bound anticoagulant mechanisms, in particular the protein C system, is shut-off by pro-inflammatory cytokines. Cytokine-mediated downregulation of thrombomodulin on endothelial cells appears to be a key phenomenon in this respect. In addition, fibrin removal is severely inhibited, due to inactivation of the fibrinolytic system, caused by an upregulation of its main inhibitor, plasminogen activator inhibitor type 1 (PAI-1). Increased fibrin formation and impaired removal leads to (micro)vascular thrombosis, which may result in tissue ischemia and subsequent organ damage. Interestingly, coagulation may have a profound modulatory effect on inflammatory pathways as well. In addition, recent studies point to an important role of metabolic factors, such as lipoprotein metabolism or glucose/insulin regulation, on the bidirectional interaction between inflammation and coagulation. Strategies aimed at the inhibition of coagulation activation may theoretically be justified and have been found beneficial in experimental and clinical studies. These strategies comprise inhibition of tissue factor-mediated activation of coagulation or restoration of physiological anticoagulant pathways.

**O3 GETTING INTO THE FLOW: RED CELLS GO ON A ROLL**

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There is a tremendous literature on red blood cell deformability, i.e. on the remarkable cell ability to change its shape in response to an external force and to pass through the narrowest blood capillaries and splenic sinuses. Cell deformability is postulated to be a major determinant of impaired perfusion, increase of blood viscosity and occlusion in microvessels. Current deformability tests like ectocytometry measure global parameters, related to shape changes at the whole cell scale. Despite strong advances in our understanding of the molecular organization of red blood cells, the relationships between the rheology of each element of the cell composite structure, the global deformability tests and the cell behavior in microflows are still not elucidated. We describe recent advances in the description of the dynamics of red blood cells in shear flow and in the mechanistic understanding of this dynamics at the scale of the constitutive rheological and structural elements of the cell. These developments could open up new horizons for the determination of red blood cell mechanical parameters by analyzing their motion under low shear flows.

**O5 BEREND HOUWEN LECTURE: CLASSIFICATION AND MONITORING OF HEMATOPOIETIC NEOPLASIA IN THE GENETIC ERA**

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**Introduction:** The identification of hematopoietic disease is dependent on the recognition of characteristics that distinguish the diseased state from normal, while disease classification relies on the ability to group patients based on common features. The tension between taxonomic fusion and fission fundamentally impacts the way we approach laboratory evaluation and ultimately the design of therapeutic intervention. As laboratory testing evolves from immunophenotypic approaches that reflect integration of cellular information to current molecular methods capable of highly multiplexed but uncorrelated assessment of large numbers of allelic variants, the result is increasing fragmentation of disease categories with the potential for significant loss of taxonomic coherence. Such a vision of personalized medicine where each patient has a unique disease state at the molecular level presents a very real challenge to the design of therapeutic strategies and the conduct of clinical trials. **Methods:** Immunophenotypic approaches to disease recognition rely on the fact that normal populations of hematopoietic cells exhibit consistent patterns of protein or antigen expression within a particular lineage as they transit from immature to mature stages of differentiation. The
observed consistency of immunophenotype for normal populations implies a high degree of genetic regulation of the maturational process. Neoplastic transformation appears to be the result of acquisition of multiple genetic mutations in pathways that control cellular proliferation, death, self-renewal, and maturation. The resulting alterations in genetic regulation give rise to changes in the expression of protein that can be used to reliably distinguish normal from neoplastic cells when considered in the context of a particular cell lineage and maturational stage. In addition, not all possible types of antigenic variation are equally represented, resulting in the ability to group cases based on similarity. This implies that despite the significant genetic variability observed within defined disease groupings, there appear to be multiple pathways to a similar integrated abnormal immunophenotype. This may in part reflect the constraints imposed by the cell type in which the mutations are acquired and suggests that context remains important for understanding the biology of these diseases. Results: The activity of diagnosis and classification in part represents an attempt to identify features of disease that predict response to therapy and outcome. While differences in outcome are apparent between different disease subtypes in aggregate, there exists notable variation within an individual disease type so that prediction of therapeutic response for an individual lacks accuracy. It may ultimately be useful to predict response prior to therapy based on pretreatment findings, but it is the direct observation of therapeutic response following therapy (minimal residual disease, MRD) that has been increasingly recognized as the most powerful predictor of eventual outcome. The data supporting the importance of MRD is most well developed for pediatric acute lymphoblastic leukemia, but similar data exist for a variety of disease states such as, adult acute lymphoblastic leukemia, acute myeloid leukemia, chronic lymphocytic leukemia, breast carcinoma, etc. Techniques for MRD assessment are now incorporated into most clinical trials for acute leukemia with flow cytometry being most commonly used in North America and PCR being more common in Europe, at least for certain disease subtypes. While flow cytometry is attractive as a technique due to its relatively low cost and rapid turn-around time, issues of standardization loom large and may ultimately limit its widespread use. PCR-based methods offer increased sensitivity of roughly 1 log in comparison to flow cytometry, but are challenged by either complex workflow resulting in expense and delayed turnaround time in the case of acute lymphoblastic leukemia or lack of universal applicability in the case of acute myeloid leukemia. Approaches using next-generation sequencing (NGS) offer the possibility of high sensitivity, assay standardization, and reasonable turnaround time, and our laboratory and others are aggressively looking to exploit this technology for MRD detection. Conclusions: The advent of NGS and the resulting explosion of information regarding the mutational landscape of cancer raise important questions regarding current strategies for both the diagnosis and monitoring of hematopoietic disease. The demonstration of clonal subpopulations within a given neoplasm and alteration of the mutational frequencies by therapeutic manipulation suggest that bulk assessments of population characteristics or frequency may not be adequate to either predict or monitor outcome. Simultaneous correlated assessment of multiple genetic mutations at the single cell level offers the most direct way to address this combinatorial problem, and efforts are underway in multiple laboratories to develop techniques capable of answering this challenge.

O6 MOLECULAR DIAGNOSIS OF PORPHYRIAS

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Introduction: The inborn errors of heme biosynthesis, the porphyrias, are eight genetically distinct metabolic disorders that result from mutations in the genes that encode the eight enzymatic steps in the conversion of succinyl-CoA and glycine to heme. These diseases can be classified as 1) “acute hepatic” (autosomal dominant Acute Intermittent Porphyria, Hereditary Coproporphyria, and Variegate Porphyria, and autosomal recessive Aminolevulinate Dehydratase Deficient Porphyria); 2) “hepatic cutaneous” (Porphyria Cutanea Tarda); and 3) “erythropoietic cutaneous” (autosomal recessive Congenital Erythropoietic Porphyria and Erythropoietic Protoporphyria, and X-Linked Protoporphyria). Recent advances in understanding their pathogenesis and molecular genetics have led to improved diagnosis and treatment. These advances include DNA-based diagnoses for all the porphyrias and genotype/phenotype correlations for certain diseases. Previously, the biochemical diagnoses of each porphyria was made by quantitation of the porphyrin precursors, 5-aminolevulinic acid (ALA) and porphobilinogen (PBG), or the total or individual porphyrin intermediates in erythrocytes, urine and/or feces. ALA and PBG have been measured by anion exchange chromatography for decades, or more recently by mass spectroscopy. The porphyrin intermediates that accumulate in their respective porphyrias can be separated and quantitated by HPLC with fluorescent detection. However, the biochemical diagnoses are limited since 1) the three autosomal dominant acute hepatic porphyrias can only reliably diagnosed during an acute attack when the ALA and PBG are elevated, since asymptomatic heterozygotes have normal ALA and PBG levels, 2) heterozygotes for the autosomal recessive and X-Linked disorders can not be detected, and 3) disease severity does not necessarily correlate with the level of substrate accumulation. With the isolation and characterization of all the human heme biosynthetic genes and their cDNAs, the mutations causing each of these disorders have been identified. Each disease results from loss-of-function gene mutations that reduce or abolish the activity of the encoded respective enzyme, with the exception of X-Linked Protoporphyria in which gain-of-function mutations in the erythropoietic-specific 5-aminolevulinate synthase (ALAS2) cause the disease. The reported mutations for each of the porphyrias are listed (and periodically updated) in the Human Gene Mutation Database (www.hgmd.cf.ac.uk see table below). For each disease, multiple gene mutations have been identified, evidencing the molecular genetic heterogeneity underlying each porphyria, and accounting in part for their relative clinical and biochemical severity. Moreover, the identification of a gene mutation in a symptomatic patient with an acute hepatic porphyria permits the identification of the asymptomatic relatives for counseling and avoidance of the precipitating factors that cause the life-threatening acute neurologic attacks. For the erythropoietic porphyrias, and especially Congenital Erythropoietic Porphyria, genotype/phenotype correlations can identify which patients will be transfusion-dependent and would be effectively cured by a successful hematopoietic stem cell transplant. Thus, the availability of heme gene diagnostic testing for these inborn errors provides diagnostic confirmation of symptomatic and asymptomatic patients, prenatal diagnosis for the severe porphyrias, and genotype/phenotype correlations to identify more severe patients who would benefit from early intervention.

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The last four decades have seen dramatic advances in deciphering the cytogenetic and molecular lesions underlying the pathogenesis of acute myeloid leukaemia (AML). These findings have not only afforded greater insights into disease biology, but also provided useful information predicting the likelihood of any given patient achieving and maintaining remission following conventional chemotherapy, leading to the development of risk-stratified treatment approaches. However, following the application of high throughput technologies it has become apparent that AML is highly heterogeneous at the molecular level. This presents a major ongoing challenge to define the individual genetic abnormalities or combinations of markers that provide significant independent prognostic information and establish their respective relationships to other pre-treatment characteristics known to influence outcome (e.g. age, presenting WBC, secondary disease). Gaining robust information on likely risk of relapse in each cytogenetically and molecularly defined subset of AML is critical to inform decisions concerning optimal consolidation therapy, including the role of allogeneic transplant. Therefore, there has been considerable interest in the development of 1) multiparameter flow cytometry, identifying leukaemia-associated aberrant phenotypes and leukaemia stem cell populations, 2) real-time quantitative polymerase chain reaction (RT-qPCR) assays detecting leukaemia-specific targets (e.g., fusion gene transcripts, NPM1 mutation) or overexpressed genes (e.g., WT1) and 3) next generation sequencing (NGS)-based approaches to provide a more precise measure of disease response. **Methods:** Minimal residual disease (MRD) assessment using flow cytometry or molecular techniques can be used to assess kinetics of disease response, which has been shown to provide independent prognostic information and is now being used to inform risk-stratified treatment approaches. Although MRD assessment at early timepoints enhances prediction of disease outcome it lacks the capacity to precisely pinpoint which patients are destined to relapse following frontline therapy and those who will be cured. This however, is feasible in patients with a leukaemia-specific molecular marker, using RT-qPCR assays which have the potential to detect 1 leukaemia cell in $10^5$-10$^6$ normal bone marrow cells. We have established proof of principle in acute promyelocytic leukaemia (APL), showing that serial monitoring of PML-RARA fusion transcripts by standardized RT-qPCR assays can be used to develop more individualised treatment approaches, guiding pre-emptive molecularly-targeted therapy with arsenic trioxide (ATO), which leads to a significant reduction in frank relapse rate and improved survival. In paediatric APL, MRD monitoring is being used by the I-BFM group to guide risk-adapted therapy that entails a significant reduction in anthracycline exposure, thereby potentially reducing the risk of cardiac toxicity (ICC-01 study). Molecular diagnostics and monitoring have also played a critical role in recent adult trials which have shown that APL can be cured with largely outpatient therapy involving ATRA+ATO without any chemotherapy. **Conclusions:** While MRD monitoring to assess remission status is now considered a standard of care in APL, as recognised in the European LeukemiaNet guidelines, its role in other subsets of AML remains to be established. However, a number of studies using RT-qPCR assays for various leukaemia-specific targets have clearly demonstrated that sequential MRD monitoring can provide independent prognostic information and predict impending relapse. We are now formally investigating within the National Cancer Research Institute (NCRI) AML17 trial whether MRD detection is clinically useful, allowing more informed choices concerning the role of allogeneic transplant and leads to meaningful improvements in outcome. Importantly, recent significant advances in molecular profiling using targeted sequencing now allow the detection of pathogenic mutations in virtually every case of newly diagnosed AML, thereby providing potential leukaemia-specific targets for MRD detection using high throughput sequencing approaches. This represents a major step forward allowing the prospect of development of more personalised approaches to the management of AML to be realised.

O9
**CLINICAL ASPECTS OF THE TREATMENT WITH DOACS**
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For more than half a century vitamin K antagonists (VKA) have been used as oral anticoagulants to prevent and treat thromboembolism. However, limitations, side effects, complications and inconvenience with VKAs meant that development of new oral anticoagulant drugs aiming for the ‘ideal’ oral antithrombotic drug profile was always desirable. The ‘ideal’ drug would be one characterised by dissociation of the antithrombotic and anticoagulant effects, in other words an antithrombotic that did not cause bleeding. So far a drug with this profile has not been realised. However, the new oral active site-specific inhibitors of coagulation serine proteases do offer an improvement over VKAs by virtue of: predictable dose responses; no need for routine monitoring; reduced need for dose adjustment; no food interactions; limited drug interactions. These direct inhibitors of serine proteases have been produced by structure-guided design and both inhibitors of thrombin and factor Xa have now been shown to be selective, orally
active, safe and at least as effective as warfarin in clinical studies. Dabigatran etexilate, a prodrug of the direct thrombin inhibitor dabigatran, rivaroxaban, apixaban and edoxaban have completed phase III studies in patients with atrial fibrillation and venous thromboembolism. Recent, meta-analyses have identified some differences in efficacy and safety indicating that the drugs may not be universally characterised as a generic group. When prescribing consideration should be given to:

- relative efficacy and safety;
- measuring and monitoring treatment;
- renal function; co-prescribing;
- management of interruption of therapy and ‘bridging’;
- management of bleeding and reversal strategies.

When commencing treatment patient education is as important as with any anticoagulant drug and clinicians should consider using a checklist.

O10
LABORATORY TESTING FOR DOACS AND TEST INTERFERENCES
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Routine tests of blood coagulation, such as the prothrombin time (PT), activated partial thromboplastin time (aPTT), and thrombin time (TT), are frequently ordered to assess the clotting function in patients. The correct interpretation of these in vitro test results in context to the ex vivo influence of anticoagulant drugs and the in vivo haemostatic system of the individual patient is dependent of the doctors’ clinical and laboratory experience. In the first part of this presentation the laboratory interference of the direct oral anticoagulants (DOAC) dabigatran, rivaroxaban and apixaban on coagulation parameters and the potential impact on test interpretation will be detailed. To improve the treatment with coumarin derivatives a standard method for reporting the prothrombin time has been assessed to ensure the proper dosage intensity that decreases the risk of bleeding while maintaining the therapeutic efficacy of the vitamin K antagonists. Chromogenic tests, such as anti-factor Xa assays, are used to monitor therapy with heparins. The target specific inhibitors dabigatran, rivaroxaban and apixaban represent a new class of anticoagulants that are administrated orally using fixed doses without the routine need of laboratory-guided dose adjustment. However, some patients’ cohorts will benefit from monitoring DOAC therapy or from determination of accurate plasma concentrations. In the second part of this presentation the potential of several methods for measuring the anticoagulant effect of the DOAC will be discussed.

O11
CLINICAL CASES AND LABORATORY TESTING OF DIRECT ORAL ANTICOAGULANTS
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The introduction of direct oral anticoagulants into clinical practice is a welcomed advance in patient care. In general, the efficacy and safety of oral factor IIa and Xa inhibitors are equivalent or superior to warfarin or low molecular weight heparins for stroke and DVT prophylaxis and treatment of venous thromboembolic events based on multiple large randomized clinical trials. Regulatory agencies in Europe and North America have approved three DOACs for similar indications without requirements for therapeutic drug monitoring. The transition from clinical trials to routine clinical practice for DOACs has many similarities to the history of low molecular weight heparins:

1. Predictable pharmacokinetics and excellent clinical outcomes without laboratory monitoring
2. No companion diagnostics for monitoring drug levels or anticoagulant activity
3. No rigorous determination of “therapeutic drug levels”
4. No effective antidote
5. Post-approval recognition of certain patients for whom standard dosing may not be ideal
6. Development of an infrastructure to manage unusual clinical situations using existing coagulation laboratory tests or developing new ones
7. Involvement of expert clinical panels and external quality assurance organizations to provide guidance to physicians and laboratories to optimize patient safety and health

This presentation will review the status of external proficiency testing in Europe and North America for monitoring dabigatran and rivaroxaban, highlight the current lack of sufficient data to define therapeutic levels for DOACs, and show examples of how PT and aPTT results can be used to support management of patients taking DOACs who present with acute bleeding.
O12
NEW CBC POC DEVICES
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Introduction: Point of care in the community and hospital out patients is increasing. New technologies are being developed to analyse the full range of standard CBC parameters and WBC differentials, all delivered in minutes. A novel POC haematology device, HemoScreen, developed by PixCell Medical uses a disposable cartridge with 20ul of blood. Employing Visco-Elastic focusing and flow-based optical imaging, the cells, are focused into a single plane, analysed by image-processing and classification algorithms. The HemoScreen offers the potential for a safe, reliable maintenance free method of performing complete CBC with 5-part differential, including flagging for morphological abnormalities. An ideal option for Physician offices, neonatal and adult ICU, oncology centres or even at home. Methods: The Abbott IVIS image-based technology, with blood imaging chamber, uses a single cartridge and 20ul of blood. Employing absorption spectrophotometry and image analysis it is intended to be easy to operate, maintenance free and has the potential to provide comparable data to conventional haematology instruments. It could offer a complete set of CBC parameters, 5-part differential and abnormal WBC flags, the ability to differentiate platelet clumps from white blood cells and flagging of sickle cells and certain parasitic infections. Conclusions: Both devices require independent evaluations according to ICSH and CLSI guidelines/recommendations to verify claims made by the manufacturers before they can be used in routine clinical practice.

O13
ENSURING PATIENT SAFETY THROUGH INNOVATIVE TRAINING FOR POINT OF CARE
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Introduction: Point-of-care testing (POCT) in community healthcare has been the subject of some concern in the UK since its early uptake many years ago. In contrast, POCT in acute secondary care settings is better controlled and managed with the appointment of POCT coordinators, managers and team in NHS Hospital Trusts, who ensure that issues such as training, quality control, external quality assurance and maintenance of POCT devices are all monitored and managed. In contrast POCT in the community is, in many cases, neglected and issues such as QC, EQA and training are left to the body that is ‘first through the door’ which is usually the device vendor. The training programme is not standardised to a nationally agreed level of competence and so variability of training and competency is widespread. There are examples of good practice where, for example, local POCT managers from nearby NHS Trusts have intervened but this is all too rare. Methods: The Metro-POCT project is a two year, MMU-based, proof-of-concept initiative funded by HEIF money. The overall aim of the project is to improve patient safety by delivering expert training to the community-based POCT practitioners. The project has made significant progress in gaining an understanding of market needs and learning needs and has begun the development of learning materials. E-learning will form a significant element of the training offering from this project and the Digi-Lab group within MMU are currently bringing to life the training material that we have created to enable training through e-learning modules and applications. Results: As part of the project, the training materials will be used as an intervention on students and healthcare professionals and so their competency will be assessed both before and after receiving training. Point-of-care testing pilot sites will be set up in the Greater Manchester primary care community to assess both practitioner and patient satisfaction in comparison to the normal central laboratory run system. Conclusions: The presentation will offer an update on community POCT attitudes and uptake in the UK, the latest survey findings from a lengthy research programme amongst community healthcare practitioners and an example of the latest e-learning developments.

O14
POINT OF CARE TESTING AT THE COMMUNITY LEVEL IN EASTERN AFRICA
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Introduction: Point of care testing (POCT) describes diagnostic testing performed at or near the site of care to obtain immediate test results to guide interventions. Improved access to diagnostic testing is part of the global effort to combat major diseases and provide better quality of care, especially to populations in low and middle income countries which have limited access to laboratory services and difficult referral systems. Methods: Automated and centralised laboratory systems in developed countries are impractical and cost prohibitive in developing countries, where most people live in rural areas with limited communication and transport systems. Major challenges facing decentralised laboratory systems in resource-poor settings include shortages of skilled personnel, inadequate infrastructure, lack of equipment and equipment maintenance, inadequate supply-chain management, and poor quality assurance. Clinicians have long distrusted the validity of laboratory testing under these conditions, and have resorted to clinically-based diagnosis and management. Widespread syndromic management results in under- and over-diagnosis and irrational treatment, which has contributed to antimicrobial resistance and drug toxicity. Results: The purpose of POCT is to provide rapid disease confirmation, accelerate treatment initiation and reduce mortality. Point of care tests must be relevant to the services offered; use no equipment or basic equipment requiring little maintenance; use robust supplies; and be user-friendly, amenable to quality monitoring and affordable. In East Africa, primary health clinic laboratories have for many decades provided services that meet POCT criteria. These laboratories traditionally conduct basic tests including microscopy on blood, stool, urine and sputum; and testing for antigens, antibodies and simple biochemical markers for syphilis, pregnancy, blood grouping and urine chemistry. Although accessible and rapid, these services are labour intensive, require trained staff and a minimum infrastructure, and the quality of testing and clinicians’ use of the services is often unknown. Conclusions: An Essential Laboratory Programme pilot study conducted in health centres in Kenya from 1992 – 1994 assessed the feasibility of peripheral laboratories conducting basic tests and their clinical usefulness [1]. Sixty two percent (62%) of outpatients required laboratory testing, and diagnosis and treatment were changed in 45% of tested patients (21% of all outpatients). On average, 40 manual tests were performed per working day, which is considered optimum for laboratory staff performing manual tests. However, quality of laboratory testing monitored through a basic external quality assessment scheme showed performance below internationally accepted levels for participation in
O15
STEM CELLS IN HEMATOLOGIC DISEASE
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The intestinal epithelium is the most rapidly self-renewing tissue in adult mammals. We originally defined Lgr5 as a Wnt target gene, transcribed in colon cancer cells. Two knock-in alleles revealed exclusive expression of Lgr5 in cycling, columnar cells at the crypt base. Using an inducible Cre knock-in allele and the Rosa26-LacZ reporter strain, lineage tracing experiments were performed in adult mice. The Lgr5+ crypt base columnar cells (CBC) generated all epithelial lineages throughout life, implying that it represents the stem cell of the small intestine and colon. Similar observations were made in hair follicles and stomach epithelium. Methods: Single sorted Lgr5+ stem cells can initiate ever-expanding crypt-villus organoids in 3D culture. Tracing experiments indicate that the Lgr5+ stem cell hierarchy is maintained in these organoids. We conclude that intestinal crypt-villus units are self-organizing structures, which can be built from a single stem cell in the absence of a non-epithelial cellular niche. The same technology has now been developed for the Lgr5+ stomach stem cells. Results: Intestinal cancer is initiated by Wnt pathway-activating mutations in genes such as APC. As in most cancers, the cell of origin has remained elusive. Deletion of APC in stem cells, but not in other crypt cells results in progressively growing neoplasia, identifying the stem cell as the cell-of-origin of adenomas. Moreover, a stem cell/progenitor cell hierarchy is maintained in early stem cell-derived adenomas, lending support to the “cancer stem cell” concept. Conclusions: Fate mapping of individual crypt stem cells using a multicolor Cre-reporter revealed that, as a population, Lgr5 stem cells persist life-long, yet crypts drift toward clonality within a period of 1-6 months. Lgr5 cell divisions occur symmetrically. The cellular dynamics are consistent with a model in which the resident stem cells double their numbers each day and stochastically adopt stem or TA fates after cell division. Lgr5 stem cells are interspersed between terminally differentiated Paneth cells that are known to produce bactericidal products. We find that Paneth cells are CD24+ and express EGF, TGF-α, Wnt3 and the Notch ligand Dll4, all important for Paneth cell function. Genetic removal of Paneth cells inhibits intestinal organoid formation. This Paneth cell requirement can be substituted by a pulse of exogenous Wnt. Results: Deletion of APC in stem cells, but not in other crypt cells results in progressively growing neoplasia, identifying the stem cell as the cell-of-origin of adenomas. Moreover, a stem cell/progenitor cell hierarchy is maintained in early stem cell-derived adenomas, lending support to the “cancer stem cell” concept. Conclusions: Fate mapping of individual crypt stem cells using a multicolor Cre-reporter revealed that, as a population, Lgr5 stem cells persist life-long, yet crypts drift toward clonality within a period of 1-6 months. Lgr5 cell divisions occur symmetrically. The cellular dynamics are consistent with a model in which the resident stem cells double their numbers each day and stochastically adopt stem or TA fates after cell division. Lgr5 stem cells are interspersed between terminally differentiated Paneth cells that are known to produce bactericidal products. We find that Paneth cells are CD24+ and express EGF, TGF-α, Wnt3 and the Notch ligand Dll4, all important for Paneth cell function. Genetic removal of Paneth cells inhibits intestinal organoid formation. This Paneth cell requirement can be substituted by a pulse of exogenous Wnt. Genetic removal of Paneth cells dramatically improves organoid formation. This Paneth cell requirement can be substituted by a pulse of exogenous Wnt.

O16
A NEW STRATEGY FOR AUTOMATIC IDENTIFICATION OF ATYPICAL LYMPHOID CELLS FROM PERIPHERAL BLOOD CELL IMAGES
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Introduction: Morphological analysis and classification of peripheral blood (PB) images to support medical diagnosis is being the subject of active research. Atypical lymphoid cells are the most difficult and challenging pathological cells to classify using morphology features only. The main objective of this work was to design a new methodology in order to achieve a robust and accurate automatic classification of atypical lymphoid cells using PB cell digital images. Methods: We analyzed 1834 digital images of individual lymphoid cells from PB films stained with May–Grünewald-Giemsa and obtained in the CellVision DM96. Among them, 180 images belong to healthy patients (N), 301 to patients with Hairy Cell Leukemia (HCL), 542 to patients with Chronic Lymphocytic Leukemia (CLL), 401 to patients with Mantle Cell Lymphoma (MCL), 334 to Follicular lymphoma (FL) and 75 B-cell Prolymphocytes (BPL). We implemented clustering of color components and Watershed Transformation to segment the nucleus, the cytoplasm and the peripheral cell region. We extracted 1429 features from these regions: 1352 color and texture features from CMYK color space, 76 geometric features and 1 from the external profile of the cytoplasm. Afterwards, we applied information theory feature selection in order to choose the best 18 features. These were used by the supervised learning algorithm Support Vector Machine (SVM) with a radial basis function kernel to classify the different subtypes of lymphocytes. Results: We performed a 10 fold cross-validation of SVM. This technique randomly divides the data set into 10 equal size subsets. A single subset is used as the validation date, while the remaining data are used for training. Then, the process is repeated 10 times, with each subset as the validation data. The accuracy of this procedure was 97.98 %. Figure 1 shows the sensitivity and specificity for each lymphoid subtype in the entire validation process. Conclusions: Our strategy includes a robust segmentation method, a complete feature extraction and a successful classification procedure. It is important to remark the high number (6) of groups involved in the classification. The use of this methodology may be a significant support tool for initial lymphoid neoplasms diagnosis from peripheral blood morphologic analysis.

O17
DETECTION OF MALIGNANT CELLS IN SEROUS BODY FLUIDS BY COUNTING HIGH-FLUORESCENT CELLS ON THE SYSMEX XN 2000
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Introduction: The body fluid module of the Sysmex XN 2000 analyzer differentiates cells into mononuclear and polymorphonuclear white blood cells (WBC) and high-fluorescent cells (HFC). In serous fluids, HFC could represent mesothelial cells, macrophages or tumour cells. The aim of our study was to evaluate the performance of the HFC count for detecting malignant cells. Methods: One hundred and sixteen serous fluids (37 ascites,
18 chronic ambulatory peritoneal dialysis (CAPD) and 61 pleural fluids) were analyzed on the Sysmex XN body fluid module. HFC were automatically measured and expressed as relative count (HFC/100 WBC) as well as absolute count (HFC/µl). All samples were microscopically screened on cytospin slides for the presence of tumour cells. Receiver operating characteristics (ROC) curve analysis was performed to determine diagnostic accuracy of HFC count to detect malignancy. Results: Tumour cells were found in 24 (14 pleural, 10 ascites and 0 CAPD fluids) out of 116 samples (prevalence 20.7%). Median relative HFC count was 13% (Inter Quartile Range [IQR] 5.8-20.3%) in malignant samples versus 1.4% [IQR 0.3-11.2%] in non-malignant samples. Median absolute HFC count was 74/µl [IQR 38-149/µl] in the malignant group versus 6/µl [IQR 1-33/µl] in the non-malignant group. Areas under the ROC curve for relative and absolute HFC count were 0.75 [95% CI 0.67-0.84] and 0.86 [95% CI 0.80-0.93], respectively. [fig. 1] A level of 100% sensitivity was reached at a cut-off value of 1.8 HFC% for the relative count and 20 HFC/µl for the absolute count, which resulted in specificities of 53% and 72%, respectively. Conclusions: ROC curve analysis showed that the presence of malignant cells in serous fluids is more accurately detected by absolute number of HFC, which is more specific than relative count. Our results indicate that malignancy can safely be ruled out if HFC count is below 20/µl. Further investigation on a larger number of samples is needed to see if this cut-off can be fine-tuned, depending on the sample type. As body fluid analysis will probably be more performed on automated analyzers in the future, HFC count could be valuable to select samples needing microscopic review.

Morphologic competency of laboratory technologists who perform bone marrow aspirate (BMA) differentials is necessary for optimal patient care. This is analogous to competency now in place for peripheral blood smears. To evaluate competency, in 2010 the CAP developed a virtual BMA proficiency testing survey using high-resolution whole slide scanning of BMA smears. Results from the first 4 years of this survey are presented. Methods: The CAP proficiency testing survey is composed of a patient clinical history, a virtual BMA smear slide with 5 cells selected for participant identification, and several disease-specific questions. BMA slides and cells for identification are carefully chosen and vetted by an expert panel of hematopathologists on the CAP Hematology and Clinical Microscopy Resource Committee (18 pathologists, 1 medical technologist). Survey participants are required to identify the 5 cells, perform a differential cell count, and answer provided questions. Participant results are collected and the mean, median, standard deviation (SD), coefficient of variation (CV) and lowest and highest values are determined for each cell type reported in the differential. Percentages of correct and alternative answers are calculated for the 5 cell identifications and questions. The summary data, together with a case discussion written by a committee hematopathologist, is provided to all participants. Results: From 2010 to 2013, a maximum of 320 laboratories participated in each of 8 unique surveys. Review of collected data demonstrate that most cells within the differential count had a SD of <5%. Cell types with a SD >5% included neutrophils (N=4), erythroid precursors (N=4), lymphocytes (N=3) and abnormal increased cell populations (e.g. blasts, promyelocytes, and plasma cells (each N=1)). Participants scored >90% on 30 of 40 cell identifications; 5 identifications had 80-89% participant consensus and 5 had <80% consensus. Less than 90% consensus was seen in the differentiation between promyelocytes, myelocytes and metamyelocytes, identification of abnormal plasma cells, identification of myeloid dysplasia, and differentiation between early erythroid precursors and myeloblasts. Laboratories successfully answered a majority of the questions. Conclusions: The virtual BMA survey accurately reproduces the experience of evaluating a BMA smear. Participants frequently had >90% consensus in identifying preselected cells, and most cells within the differential count had a SD <5%. Participants are able to compare their individual differential count and cell identifications to data collected from all participating laboratories. This survey augments laboratory proficiency testing in BMA smears by providing an effective tool for evaluating competency of individual technologists.

O18 PROFICIENCY TESTING FOR BONE MARROW ASPIRATE SMears USING VIRTUAL SLIDES, A COLLEGE OF AMERICAN PATHOLOGISTS EXPERIENCE.

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1College of American Pathologists, Hematology and Clinical Microscopy Resource Committee Northfield, IL, USA, 2Virginia Commonwealth University Richmond, VA, USA, 3Emory University Atlanta, GA, USA, 4University of New Mexico Albuquerque, NM, USA, 5University of Utah Health Sciences Center Salt Lake City, UT, USA, 6Sutter Health Shared Laboratory Livermore, CA, USA

O19 CELL-BY-CELL ASSESSMENT OF MEDIAl MICROSCOPE (MM) AS A BETWEEN-OBSERVER TOOL FOR HARMONIZATION AND PROFICIENCY TESTING (ON BEHALF OF WP10 LEUKEMIAnET DIAGNOSIS MORPHOLOGICAL FACULTY)

Gina Zini1, Marie C. Béné2, Ombretta Barbagallo1, Giuseppe d’Onofrio1
1Catholic University of Sacred Heart, Rome, Italy, 2University Hospital, Nantes, France

Introduction: Medial Microscopy (MM) with whole smear imaging is based on a robotic scanner microscope that digitalizes an entire glass slide smear. It is validated for histopathology, teleconsulting, teaching and research. We report a pilot study, started within the European LeukemiaNet Diagnosis WP10 morphological faculty, to assess interobserver agreement in “morphologically critical” cell classification using MM, for application in quality assurance and proficiency testing. Methods:
A digitalized smear from bone marrow aspirate of an untreated acute monocytic leukemia patient was submitted to 16 multi-country hematologists expert in cytomorphology. Consensus agreement on 110 cells was verified at the 75% level (≥12/16 observers providing identical cell classification) and 60% level (>9/16 observers), for all cells and identification of: - all blasts (present: ≥12/16 participants; absent: <5/16; disagreement: 5-11/16); - blastic nature of the 63 cell assigned to the monocyte lineage (any type) by more than half of the participants. Results: A 75% agreement was reached for 71/110 cells (65%). A 60% agreement was reached for 92/110 cells (84%); an agreement <60% was obtained only in 16% of the cells. Agreement was 100% for lymphocytes and erythroblasts; 17% of the cells, mainly damaged, were considered morphologically unclassifiable by at least 12 observers. Agreement on the definition of a cell as “blast” (including monoblasts, promonocytes, myeloblasts and unspecified, as per the 2008 WHO classification) or “non-blast” was 91/110 cells (83%). Agreement on the “blastic” nature of cells of mononuclear lineage was also excellent (Table, Figure). Table. Examples of agreement and disagreement in the cytomorphological classification for cells of the monocytic lineage with (D05, D10, D17) or without agreement (D106, D107).

Participants that identified the cell as:

<table>
<thead>
<tr>
<th>Cell</th>
<th>Mono-blast</th>
<th>Promonocyte</th>
<th>Myeloblast</th>
<th>Atypical monocyte</th>
<th>Mature</th>
<th>Blast (any type)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D05</td>
<td>14</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>15/15</td>
</tr>
<tr>
<td>D10</td>
<td>5</td>
<td>9</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>14/15</td>
</tr>
<tr>
<td>D17</td>
<td>4</td>
<td>11</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>15/16</td>
</tr>
<tr>
<td>D106</td>
<td>0</td>
<td>6</td>
<td>5</td>
<td>4</td>
<td>1</td>
<td>11/16</td>
</tr>
<tr>
<td>D107</td>
<td>0</td>
<td>7</td>
<td>4</td>
<td>4</td>
<td>0</td>
<td>11/16</td>
</tr>
</tbody>
</table>

Figure. Agreement on blastic nature of mononuclear cells. Cell number versus participants who have classified each cell as monoblast/promonocyte or atypical-immature monocyte/mature monocytes. Conclusions: Agreement on cell classification and definition of blast cells, monoblasts and promonocytes was excellent. The main source of discrepancy resided in identification of promonocytes/atypical monocytes. MM has good potential for standardization and harmonization in cytomorphological diagnosis, quality assurance and proficiency testing. Pantanowitz. Arch Pathol Lab Med 2013, 137:1710-1722.

O20 INITIAL PROFILE OF POSITIVITY AND PERSISTENCE OF ANTIPHOSPHOLIPID ANTIBODIES AFTER A PERIOD OF TWELVE WEEKS

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Introduction: Some forms of the antiphospholipid antibodies syndrome (APS) require early diagnosis, without waiting for confirmation of biological assays at least 12 weeks later, as required by Sapporo/Sydney classification criteria. The objective of this work was to define which initial laboratory criteria are associated with the persistence of APL at an interval of at least 12 weeks after the first positive sample. Methods: APL assays were the following: PTTLA® (Diagnostica Stago®), DRVVT LA® (Siemens), anti-cardiolipin (ACL) ELISA IgG and IgM (European Forum on antiphospholipide antibodies) and anti-β2-GPI ELISA IgG and IgM NOM (Orgentec®). The cutoff for the positivity were: 1.2 for the ratio (Mixing Patient (P) + Control(C)/C) for PTTLA® and screening/confirm ratio (P/C) for DRVVT of coagulation tests, the 99th percentile (14 GPL; 6 MPL) for ACL ELISA and anti-β 2-GPI ELISA (8 U/µL, for both isotypes). A relational database (APL-DB) from 2005 to 2013 (7963 patients) has allowed retrospective analysis of APL data and their evolution at 12 weeks and later by 2 approaches: (1) decision analysis to identify the characteristics of assays predictive of persistent positivity (WEKA data mining), and (2) statistical analysis: i) c² test to compare frequencies of the initial classes of positivity (Class I : multiple positivity, Miyakis et al, 2006) between patients who remain positive and those who will not negate at 12 weeks ii) Studenttest to compare means of the assays values at the first sample between these two groups of patients. Results: The decision trees show that a result greater than a ratio of 1.33 and 1.5 for respectively PTTLA and DRVVT, a value of 15 GPL for IgG ELISA ACL, and 6 MPL for IgM were associated with the persistence of antibodies at least 12 weeks. The frequency of patients initially positive and persistent at least 12 weeks was higher in class I than in the other classes. All patients of class I at the first sample remained positive beyond 12 weeks. In addition, in all classes, the initial numerical values of the tests at the first sample were significantly higher when they persist at least 12 weeks. Conclusions: This study has identified profiles of positive tests at the first sample, which were associated with persistence of positivity beyond 12 weeks. The cutoffs of each test, associated with this persistence, were established by data mining. If these results are confirmed by other teams, they could allow an earlier diagnosis of APS.
A NEW HIGHLY SELECTIVE FACTOR XIIa INHIBITOR BASED ON INFESTIN-4

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1Laboratory for Molecular Mechanisms of Hemostasis, Center for Theoretical Problems of Physicochemical Pharmacology Moscow, Russia, 2Research Division, Federal Research and Clinical Center of Pediatric Hematology, Oncology and Immunology Moscow, Russia, 3Laboratory of mammalian cells bioengineering, Centre “Bioengineering” Russian Academy of Sciences Moscow, Russia, 4Waksman Institute of Microbiology, Rutgers, NJ, USA, 5Research Department, HemaCore LLC, Moscow, Russia, 6Department of Physics, Moscow State University, Moscow, Russia

Introduction: Selective inhibition of activated coagulation factor XIIa (FXIIa) is promising goal for the anti-thrombotic therapy and global coagulation assays. There are numerous FXIIa inhibitors, including proteins from Z.mays (CTI, K. 0.4 nM), L.cylindrica (LCTI-III, K. 10 nM), C.maxima (CMTI-III, K. 23 nM) and T.infestans (infestin-4, K. 0.1 nM) that are poorly characterized. In addition, their FXII-independent activities (K. in range 1-50 µM towards other coagulation proteases) result in altered dynamics of fibrin formation. Here we aimed to develop more selective FXIIa inhibitor exhibiting minimal FXII-independent activity on coagulation. Methods: Infestin-4, its mutants and CMTI-III were produced in E.coli as soluble fusion proteins and purified with Ni-chelating and ion-exchange chromatography. CTI was extracted from the corn kernels and LCTI-III was chemically synthesized. The activity of these inhibitors was estimated by aPTT-elongation in normal plasma. K. values against coagulation proteases were determined in chromogenic assays. The selectivity was estimated with Thrombodynamics assay in FXII-depleted plasma by fibrin clot growth from immobilized tissue factor (TF). Results: We generated new infestin-4 mutants with amino acid substitutions at P2, P1’ and P2’ positions relatively to the Arg10 reactive site. Inf4-MutB with TRNFVA sequence at positions P2-P4 efficiently inhibited FXIIa (K. 0.9 nM). It had only minor non-specific inhibition of thrombin (K. 0.9 µM) which did not cause any shift in the dynamics of TF-initiated coagulation in FXII-depleted plasma. Inf4-MutB at 20µM caused a 3-fold elongation of aPTT, blocked contact-activated clotting in Thrombodynamics assay and prolonged the shelf-life of the whole uncitrated blood. Other infestin-4 mutants inhibited FXIIa 5-10 times less effective than Inf4-MutB. Moreover, they inhibited FIXa when fused with thioredoxin (K. 1 µM or less). CTI, LCTI-III and CMTI-III were either less effective FXIIa inhibitors (K. between 0.5 and 30 nM) or inhibited factors XIIa, FXa (K. between 1 and 30 µM) and TF-initiated coagulation in FXII-depleted plasma. <hr align="left" size="1" width="33%"/>

Conclusions: The new highly selective FXIIa inhibitor Inf4-MutB prevents an interference of contact activation with the TF-initiated coagulation as was shown by Thrombodynamics. This inhibitor can be applied for blood anticoagulation, as well as for plasma stability aid in the coagulation assays.

ADENOSINE TRIPHOSPHATE RELEASE ASSAY WITH DIFFERENT CONCENTRATIONS OF THROMBIN FOR THE ASSESSMENT OF PLATELET FUNCTION DISORDERS

Sylvie Mulliez, Katrien Devreese

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Introduction: Inherited platelet disorders constitute a heterogeneous group of bleeding. A subgroup of these disorders are characterized by deficiency in the number of granules, granule content or their release mechanisms. In many cases, these defects are associated with reduced platelet aggregation. The measurement of platelet dense granule content by lumi-aggregometry is useful in the diagnosis. Dense granules are rich in adenosine triphosphate (ATP), after activation the content is secreted, further enhancing both platelet adhesion and activation. Secretion of reduced amount of ATP is indicative of either a release defect or a storage pool disease (SPD). By increasing the concentration of the agonist, a distinction could be made between SPD (equal ATP release in both agonist concentrations) and a secretion defect (increased ATP release with higher agonist concentrations). We evaluated the usefulness of activation with different concentrations of thrombin (1 and 5 U/ml) for the detection of platelet dense granule disorders. Methods: From January 2011 to September 2013 we retrospectively evaluated the samples of patients with varying bleeding symptoms and reduced ATP secretion (<0.5 nmoles) activated with 1U/mL thrombin. On these samples ATP secretion after activation with a higher concentration of thrombin (5 U/ml) was compared. We correlated these data with results of platelet function analyzer (PFA), light transmission platelet aggregation (LTA) with several agonists and electron microscopy (EM) of the thrombocytes. Results: 73 samples showed an ATP release with 1U/mL thrombin below 0.5 nmoles. Figure 1 shows the results of the ATP secretion with 1U/ml versus 5U/ml thrombin. In only 18% of these samples the ATP secretion increased with 5U/ml thrombin. In one sample the ATP release with 5U/ml thrombin was >0.5 nmoles. We did not receive a second sample to confirm. Clinically the patient was not suspected for dense granule defect. 16 patients (25 samples) were confirmed with the diagnosis of SPD based on results of LTA, EM, ATP release and the clinical symptoms. The median of the ATP release was 0.19 and 0.00 nmoles, respectively activated with 1 and 5 U/ml thrombin. The most common impairment in LTA was observed with epinephrine (54%). The PFA and LTA with other agonist (adenosine diphosphate, collagen, ristocetine, arachidonic acid, and thromboxane analogue U46619) was impaired in 21% of the samples. Conclusions: We observed no additional advantage to perform ATP secretion with two concentrations of thrombin. In patients with SPD the impaired LTA with epinephrine is most commonly seen.
O23 USE OF INTERPRETIVE SOFTWARE IMPROVES TURNAROUND TIME AND DATA ENTRY FOR COMPLEX COAGULATION TESTING IN A HIGH VOLUME LABORATORY
Heesun Rogers, Walter Henricks, Jean Klinkenberg, Kandice Kottke-Marchant
Cleveland Clinic, Cleveland, OH, USA

Introduction: In high complexity coagulation testing, comprehensive interpretation with reflexive diagnostic algorithms by expert pathologists can reduce misdiagnosis, delayed diagnosis and inappropriate procedures/medications, and can support appropriate test selection. However, manual entry of narrative interpretation into a laboratory information system (LIS) by medical technologists (MT) can cause post-analytical errors and delay the report. Interpretive software that interfaces with LIS can facilitate standard workflow, standardized coded comments, user-friendly formatting, customized comments and sign-out, and promises to reduce medical errors and turnaround time. Authors reviewed turnaround time and conducted time studies for entering coded comments by MTs and residents/pathologists before and after implementation of interpretive software. Methods: We analyzed turnaround time of large diagnostic panels (platelet aggregation (PLTAGG), lupus anticoagulant (LA), hypercoagulability (HCOAG) and von Willebrand disease (VWD)) for random 2-month periods, and collected and analyzed time for entering coded comments into LIS in random days before (2010) and after (2013) implementation of AMEDx interpretive software (American Medical Diagnostics Inc, Fairfax, VA). Results: The turnaround time between specimen arrival in the laboratory and sign-out of the interpretation was significantly reduced in all panels (Table).

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</tr>
<tr>
<td>VWD</td>
<td>53 (227.8)</td>
<td>160.19 (165.88)</td>
<td>0.0003</td>
<td></td>
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</table>

Results: average hour (median) Times for MTs entering codes showed significant reduction from average 52.2 minutes (median 41.0) to 13.1 minutes (median 10.0) with AMEDx implementation (p<0.00001). MTs spent average 10.9% of working hours per day to enter codes before AMEDx, which was reduced to 2.7% with AMEDx implementation, spending 0.7 minutes per test (p<0.0001). Overall time for coding comments/interpretation by residents/pathologists was not significantly different before and after AMEDx implementation (average 4.3 vs 5.7 minutes, respectively, (p=0.0684), which allows formatting or editing of customized comments by patient’s clinical and medical history. Conclusions: Our data showed significant reduction of turnaround time and MT time spent entering codes with AMEDx implementation, allowing them to focus on testing itself. Using interpretive software and standardized coded comments in coagulation tests can provide accurate and timely interpretation with patient-specific interpretive comments, in pursuit of quality patient care.

O24 BLAST CELL DECREASE MONITORING IN PERIPHERAL BLOOD FROM AML PATIENTS USING MULTIPARAMETRIC FLOW CYTOMETRY AND HEMATOFL ow TM METHODS.
Kaatuar Allou1, Marie Christine Béné2, Jean-Philippe Vial1, Francis Lacombe1
1 CHU Bordeaux, BORDEAUX, France, 2 CHU, Nantes Nantes, France

Introduction: It has been reported that the chemosensitivity of patients with acute myeloblastic leukemia (AML) during induction chemotherapy could be assessed using a flow cytometry method (Leukemia. 2009; 23(2):350-7). Briefly, peripheral blood samples were tested daily for the first seven days of therapy with a multiparametric flow cytometry method allowing to positively define mature peripheral leukocytes, and thus deduce the amount of blast cells. Measuring the blast decrease rate (BDR) allows to determine either the slope of blast decrease (BDRSlope) or the time needed to achieve the clearance of 90% of peripheral blasts (BDR90). These two values correlate strongly with both the rate of remission and the probability of relapse. In this work, we compare a ten color multiparametric flow cytometry method to the HematoflowTM system for blast cell monitoring. Methods: For each blood cell samples, two immunophenotyping procedures were performed daily: (1) a ten color analysis with the following monoclonal antibody panel CD65-FITC / CD14-PE / CD13-EDC / CD33-PC5 / CD34-PC7 / CD117-APC / CD7-APC700 / CD11b/APC750 / CD19-PE / CD45-KO and (2) the CytodiffTM panel comprising CD36 FITC / CD294 PE / CD294 PE / CD19 ECD / CD16 PC5 / CD45 PC7. Blast cell percentages were obtained with a specific gating using KaluzaTM for procedure (1) and with the automatic gating provided by HematoflowTM for procedure (2). The two modalities previously described for blast cell clearance evaluation (BDRSlope and BDR90) were applied to each procedure and results were compared for each measurement couple. Results: On 49 samples from ten patients, blast cell percentages obtained with each method were perfectly correlated (r = 0.99, R² = 0.99), BDRSlope were also very well correlated (r= 0.99) and BDR90 values were exactly the same for each two methods. Limitations of HematoflowTM procedures are low number of peripheral blood blast cell percentages (< 3%) and AML with monocytic component. A prospective study is under investigation to confirm results of the reference publication (Leukemia). Conclusions: Response to chemotherapy is usually investigated in AML therapeutic trials by performing morphological examination of a bone marrow aspiration at the end of the induction period. The new approaches described here provide additional information and could be considered as very comparable if appropriate conditions are respected. The methodology is robust and could be widely disseminated using such automated procedures as Hematoflow.

O25 AN ACTIVE, COLLABORATIVE APPROACH TO TEACHING FLOW CYTOMETRY DATA INTERPRETATION SKILLS IN THE CONTEXT OF HAEMATOLOGICAL MALIGNANCIES.
Kathy Heel1, Matthew Linden2, Clayton Fragall1, Jill Finlayson1, Wendy N Erber1, Kimberley J Roehrig1
1 School of Pathology and Laboratory Medicine, The University of Western Australia, Crawley, Australia, 2 Centre for Microscopy, Characterisation and Analysis, The University of Western Australia, Crawley, Australia, 3 Department of Haematology, PathWest Laboratory Medicine, Perth, Australia

Introduction: In high complexity coagulation testing, comprehensive interpretation with reflexive diagnostic algorithms by expert pathologists can reduce misdiagnosis, delayed diagnosis and inappropriate procedures/medications, and can support appropriate test selection. However, manual entry of narrative interpretation into a laboratory information system (LIS) by medical technologists (MT) can cause post-analytical errors and delay the report. Interpretive software that interfaces with LIS can facilitate standard workflow, standardized coded comments, user-friendly formatting, customized comments and sign-out, and promises to reduce medical errors and turnaround time. Authors reviewed turnaround time and conducted time studies for entering coded comments by MTs and residents/pathologists before and after implementation of interpretive software. Methods: We analyzed turnaround time of large diagnostic panels (platelet aggregation (PLTAGG), lupus anticoagulant (LA), hypercoagulability (HCOAG) and von Willebrand disease (VWD)) for random 2-month periods, and collected and analyzed time for entering coded comments into LIS in random days before (2010) and after (2013) implementation of AMEDx interpretive software (American Medical Diagnostics Inc, Fairfax, VA). Results: The turnaround time between specimen arrival in the laboratory and sign-out of the interpretation was significantly reduced in all panels (Table).

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**Introduction:** Advances in education research have the potential to improve the way we teach methods of scientific analysis to students in higher education. Increasingly universities are using active, collaborative methods to teach students to perform scientific interpretations and understand science concepts. We developed group-based activities for students to learn skills in manipulating flow cytometry data using FlowJoX software by examining immunophenotyping results in haematological malignancies. **Methods:** Undergraduate students were given compensated clinical flow cytometry data and asked to diagnose 3 patients with different haematological malignancies on the basis of immunophenotype. Clinical data was de-identified and provided by PathWest Laboratory Medicine in the form of FCS data files. In a single lesson lasting approximately 3 – 4 hours, students were first introduced to the principles of flow cytometry, then given 3 sets of unknown patient data and asked to design a gating strategy and make a diagnosis. Reference tables were provided with details of the characteristic phenotypes of several haematological profiles. Student feedback was collected via an anonymous online survey in the days following the activity. 35 responses were received from 72 enrolled students (49%). **Results:** The active, collaborative approach allowed students to achieve learning outcomes not included in previous classes, for example having students design their own gating strategy, without forgoing essential outcomes such as interpretation of dot plots. Students found the activities engaging and informative and reported increased confidence with manipulation and interpretation of flow cytometry data. High levels of confidence correlated with positive ratings of flow cytometry and students who reported the highest levels of confidence were most likely to agree with the statement “I like flow cytometry more than other activities in the unit and might consider it as a career” (40% of respondents in the highest confidence category compared with 4% in lower confidence categories). This correlation was strongest in students with no prior exposure to flow cytometry. **Conclusions:** We designed active, collaborative activities to teach students how to manipulate flow cytometry data. This approach had numerous advantages over previous teaching activities (limited to scatter plot interpretation), including increased student engagement and learning, greater student confidence and association with flow cytometry as a career choice.

**O26**

**B-CELL CLONES FROM MULTICLONAL B-CELL CHRONIC LYMPHOPROLIFERATIVE DISORDERS SHOW MOLECULAR/CYTGENETIC FEATURES RELATED TO EARLIER DISEASE STAGES VS. CLONES FROM MONOCLONAL CASES.**

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**Introduction:** Multiclonal monoclonal B-cell lymphocytosis (MBL) and B-cell chronic lymphoproliferative disorders (B-CLPD) may consist of expansions of ≥2 B-cell clones potentially associated with chronic antigen-driven immune responses. In fact, this is particularly frequent at the earlier MBL stages (MBL+), which would further support the potential reactive nature of MBL among individuals with normal lymphocyte counts, prior to the stepwise acquisition of genetic alterations and progression to clinical MBL (MBL+) and chronic lymphocytic leukemia (CLL). If this hypothesis holds true, specific antigenic determinants could potentially be more frequently shared between the coexisting B-cell clones of monclonal cases than between the expanded B-cells in different monoclonal MBL and B-CLPD patients, due to a greater probability of interaction with common immunological determinants. This might even be true when the coexisting clones display clearly distinct immunophenotypic and cytogenetic, as well as clinical features. **Methods:** We comparatively analyzed the B-cell receptor (BCR) repertoire and the molecular profile, as well as the phenotypic, cytogenetic and hematological features of 228 CLL-like and non-CLL-like clones between monoclonal (n=85 clones from 41 cases) versus monoclonal (n=143 clones) MBL, CLL and other B-CLPD. **Results:** The absolute median number of peripheral blood CLL-like MBL1 and CLL B-cell clones were significantly lower in multclonal than in monoclonal cases. Regarding cytogenetic features, the frequency of CLL-like MBL and CLL clones from monclonal cases that showed cytogenetic alterations was significantly lower than that found among clones from monclonal cases. Likewise, the proportion of CLL-like B-cell clones showing coexistence of ≥2 cytogenetic alterations was also significantly lower in multclonal than in monoclonal cases. The molecular profile of B-cell clones from multclonal vs monoclonal cases was very similar. Of note, the BCR of B-cell clones from multclonal cases showed a slightly higher degree of HCDR3 homology than B-cell clones from monclonal cases. Based on the phylogenetic proximity of their BCR, we could further identify within the B-cell clones from multclonal cases, a considerably represented subgroup of B-cell clones showing preferential usage of IGHV3 genes and shorter HCDR3 sequences carrying a significantly higher number of IGHV mutations vs the unrelated clones. **Conclusions:** Altogether, our results suggest that multclonality is typically associated with early stages of B-CLPD, with potential involvement of multiple and diverse antigenic determinants of a common antigen, at least in a specific subset of cases.

**O27**

**MULTICOLOR FLOW CYTOMETRY FOR ASSESSMENT OF MINIMAL RESIDUAL DISEASE IN ACUTE LEUKEMIAS. TAMPERE EXPERIENCE WITH 10-COLOR PANELS.**

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**Introduction:** Minimal residual disease is an important prognostic marker in acute leukemias. The multicolor flow cytometry (MFC) is based on detection of leukemia associated immunophenotypes (LAIP) and is applicable for almost all patients with acute lymphoblastic leukemia (ALL) and for 80-90% on patients with acute myeloid leukemia (AML). The published reports, so far, have mainly applied up to six-colour panels. The new generation equipments, softwares and emerging range of directly conjugated monoclonal antibodies support the application of ten-color MFC. It offers high specificity and sensitivity also in small samples. Especially in AML, where LAIPs may be heterogeneous and phenotype shifts occur, ten-color approach is an advantage. Here we describe our MRD panels for AML and B-ALL. **Methods:** The fluorochromes used are: FL1=FITC, FL2=PE, FL3=ECD, FL4=PC5.5, FL5=PC7, FL6=APC, FL7=APC Alexa700, FL8=APC-Alexa750, FL9=BV421, FL10=KO. The AML MRD tubes are: (1) CD15, CD13, CD16, CD33, CD117, CD19, CD34, CD45, CD11b, HLADR. (2) CD34, CD64, CD56, CD33, CD117, IREM2, CD34, CD45, CD14, HLADR. (3) CD7, CD96,Clec12A,
CD45RA, CD33, CD117, CD123, CD34, CD45, CD38, HLADR. (4) CD99, CD11a, CD3, CD33, CD117, CD133, CD34, CD45, CD4, HLADR. The B-ALL MRD tubes are: (1) CD66c, CD58, CD10, CD22, CD19, CD123, CD34, CD38, CD20, CD45. (2) CD36, CD13 or NG2, CD10, CD33, CD19, CD304, CD34, CD38, CD20, CD45. We use Navios equipment and Infinicyt software for the analysis. Results: We have designed the present panels during three years. The present strategy with four tubes in AML, allows not only detect aberrant blasts, but also to evaluate the maturation of granulopoiesis and monocytes. Additionally, it is possible to identify some minor cell types which often contaminate the blast area, such as mast cells, dendritic cells and plasma cells. Besides the four standard tubes in AML, it is possible to design patient specific combinations, if informative LAIP markers are indentified at diagnosis. In B-ALL the first tube alone may be informative, but if enough sample is available, the second tube is assayed for confirmation. The aim is to collect at least 500000 events to reach the sensitivity of 0.01%. A library of normal and regenerating bone marrows is mandatory especially for AML to evaluate the specificity of LAIPs. Examples of relevant plots will be shown in the presentation. Conclusions: In conclusion we have optimized the present panels for AML and B-ALL MRD samples. The panel design has required careful planning, titration of antibodies, and compensation. We have found these panels to be cost-effective and useful for residual disease detection.

O28 APPLICATION OF DIFFERENTIAL MOLECULAR PROFILING OF HUMAN HEMATOPOIETIC TISSUES DURING HUMAN ONTOGENESIS FOR HEMOGLOBINOPATHIES THERAPEUTICS
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Introduction: Hemoglobinopathies result from genetic quantitative and qualitative abnormalities in the hemoglobin molecule production and are the commonest single gene disorders worldwide. Different types of hemoglobin molecules are produced during the embryonic, fetal and adult erythropoiesis. Fetal hemoglobin (HbF) production is expressed at high levels during the fetal stage and gradually declines in the subsequent developmental stage to reach less than 2% of total hemoglobin shortly after birth. As it has been shown from clinical observations, high levels of HbF improve the clinical symptoms of β-thalassemia or sickle cell disease (SCD) patients. In humans, the increased HbF production and the switch from fetal to adult hemoglobin are controlled by various regulatory factors, by a mechanism that still remains elusive. Methods: We have employed whole transcriptome analysis in order to identify the transcription profile of human hematopoietic tissues in different stages of ontogenesis. In particular, we have isolated and cultured ex vivo human erythroid progenitors from adult peripheral blood, umbilical cord blood and human fetal liver. Total isolated RNA from erythroid progenitor cells was labeled and hybridized to the Affymetrix Human Genome U133 array. Different expression of probe sets among the previous hematopoietic tissues was discriminated by AltAnalyze software. Results: Our comparative analysis include three different groups; umbilical cord blood versus adult peripheral blood, fetal liver versus adult peripheral blood and umbilical cord blood versus fetal liver versus adult peripheral blood. Ours results indicate that 165 genes were differentially expressed between the first group, 1239 genes were differentially expressed in the second group and finally 348 genes were differentially expressed between high (umbilical cord blood and fetal liver) and low HbF expressing tissues (adult peripheral blood), indicating that these genes may be implicated in potentiating HbF levels. Conclusions: Our data, in conjunction with our previously published observations (Borg et al., 2012, Tafrali et al., 2013), hold promise to identify unique molecular pathways involved in HbF production, which will subsequently be exploited in β-thalassemia patients’ stratification and possibly in individualization of β-type-hemoglobinopathies therapeutics.

O29 NOVEL MUTATIONS IN THE ERYTHROPOIETIN RECEPTOR (EPOR), PROLYL HYDROXYLASE DOMAIN-2 (PHD2/EGLN1) AND HYPOXIA INDUCIBLE FACTOR 2&ALPHA; (HIF2A/EPAS1) GENES AS A CAUSE OF HEREDITARY ERYTHROCYTOSIS
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Introduction: Significant advancements in the understanding of hereditary erythrocytosis have been made in recent years. Etiologies include high oxygen affinity hemoglobin variants, EPOR and oxygen sensing pathway gene mutations. We report nine novel mutations. Methods: Initial evaluation included CBC and hemoglobin analysis. Other testing included oxygen dissociation curve (p50), mass spectrometry, and erythropoietin (Epo) level. Sanger sequencing was performed on the EPOR (exon 8), PHD2/EGLN1 (exons 1-5) and HIF2A/EPAS1 (exon 12) genes, and variably on the FH (von Hippel Lindau) gene (exons 1-3).

Results: All patients presented with unexplained erythrocytosis.
portion of the catalytic domain. Due to their proximity, both of the HIF2A mutations, p.P534R and p.P544R, are postulated to interfere with hydroxylation of proline 531, a process which signals the degradation of the HIF2A protein. **Conclusions:** To our knowledge all of these mutations are previously unreported. The relatively high frequency of novel mutations suggests that those associated with erythrocytosis may be largely private to individual families.

**O30**

**AN IN VITRO MODEL TO INVESTIGATE THE PATHOLOGIC SIGNIFICANCE OF NOVEL MUTATIONS IN THE ALPHA GLOBIN GENES.**

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**Introduction:** Alpha thalassaemia is most commonly due to deletions of one or more of the alpha globin genes, however non-deletional mutations of either HBA1 or HBA2 are not infrequent, accounting for approximately 10% of cases of alpha thalassaemia in our laboratory. As a result we have included sequencing of HBA1 and HBA2 in the diagnostic algorithm for the investigation of patients suspected of having alpha thalassaemia. When novel base substitutions are identified, the reporting scientists and pathologists must assess whether these are pathogenic or polymorphic in order to provide the relevant information to the requesting clinician. In some cases it is not straightforward, and in this setting an in vitro system is useful to provide the scientists and pathologists with the relevant information to interpret the clinical significance.

**Methods:** We have developed a model using an expression vector containing the genomic alpha globin sequence in which expression of the alpha globin gene is driven by the alpha globin promoter. This may therefore be used to study mutations in the promoter region, the exonic regions, the intervening sequences, and the 3' UTR. Mutations corresponding to those identified in the clinical samples are created by site directed mutagenesis and the mutant vectors are transfected into 5637 cells. These have been selected for this model based on the criteria that they do not express alpha globin but have been demonstrated to express critical transcription factors including GATA2, NFE2, KLF1 and KLF4. Analysis of the impact of the mutations includes quantification of alpha globin mRNA, sequencing of cDNA to assess for splicing abnormalities, and immunocytochemistry using antibodies directed against the N- and C-terminal regions of the alpha globin genes to assess alpha globin expression.

**Results:** To date the following mutations have been analysed using this system: Promoter mutations: HBA2.c.-59C>T, HBA2.c.-81C>A and HBA2.c.-91G>A. Splice mutations: HBA2.c.95+1G>A, HBA2.c.94A>C, HBA2.c.94A>G and HBA2.c.301-3C>G Exonic mutations: Hb Karawara [HBA2.c.177C>G], Hb Boghé [HBA2.c.177C>A], Hb Hamilton Hill [HBA2.c.388delC] 3'UTR: HBA1.c.*+46C>A We present data on the validation of the model and experimental findings in relation to the above mutations.

**Conclusions:** This is a useful system to evaluate the pathogenic significance of novel mutations in the alpha globin genes.

**O31**

**A NEW UKNEQAS(H) DEVELOPMENT: DIAGNOSIS OF LIQUID SAMPLES FROM NEWBORNS AT RISK FOR A SIGNIFICANT HAEMOGLOBINOPATHY.**

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**Introduction:** UKNEQAS(H) provides several a suite of external quality assessment schemes for haemoglobinopathy diagnosis. This suite supports the laboratory protocols encompassing antenatal haemoglobinopathy screening; diagnostics and monitoring of affected patients; newborn dried blood spot screening and molecular diagnostics. Newborns known to be at risk for a major haemoglobinopathy require rapid diagnosis of a liquid sample but this analytical approach is not covered by any existing quality assessment scheme. In 2012, a pilot study was undertaken to assess the feasibility of such. This was followed by six further distributions from July 2013-March 2014, during which time shadow scoring was undertaken on results submitted and the scheme introduced fully from April 2014 as an option in the UKNEQAS(H) Abnormal Haemoglobins scheme. This development was devised after assessment of interest from UK laboratories and in the absence of any scheme already in existence.

**Methods:** Two specimens, both comprising of mixtures of normal cord blood and venous blood from an adult patient with HbSS disease were distributed as simulated liquid samples from newborns specifically for testing for sickle cell disease. One of each of these samples was sent as part of two successive survey distributions for the Abnormal Haemoglobins Scheme. The specimens were accompanied by brief clinical data, FBC details and a set of coded comments to assist with interpretation and reporting. The results of fraction identification and interpretation were assessed. A questionnaire sent to UK participants sought their views on the utility of the samples distributed. Outcomes showed significant support for the initiative and the pilot study was followed by six further distributions during July 2013-March 2014.

**Results:** Results of the pilot study showed that 81% of participants reported correct fraction identification for the first sample; 68% for the second and 54% of laboratories gave correct interpretation for both samples. Shadow scoring results on subsequent distributions showed incorrect fraction identification in >1% of cases but many inconsistencies and inappropriate comments within the interpretation of results.

**Conclusions:** The need for EQA to assess analysis of liquid samples from newborns has been established and introduced as part of the UKNEQAS(H) repertoire. Sample analysis was found to be very good but interpretive comments often inconsistent and inappropriate. A review of the process was undertaken and participants given direction. This has created a useful mechanism for assessment of liquid sample analysis from newborns, which was previously unavailable from any source.
potential to become standard EV detection methods in a clinical diagnostic setting. In addition to the accuracy of the detection technique, other factors such as high-throughput, cost-effectiveness, time consumption and required operator skill are important to consider. A combination of increasing fundamental knowledge, technological progress, standardization of sample collection and processing protocols is required for EVs to become reliable predictors of altered physiology or development of disease suitable for routine clinical diagnostics in the near future.

O33
ACQUIRED NEUTROPENIAS
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Acquired neutropenias mainly fall into three categories: drug-induced, autoimmune and idiopathic neutropenias. Infections are often mild in patients with autoimmune and idiopathic neutropenias (1). Neutropenia is defined by an absolute neutrophil count (ANC) below 1.5 x10^9/L and the term chronic is used when neutropenia lasts for at least three months. However, in persons of certain ethnic groups ANC below 1.5 x10^9/L is normal. The mechanisms of benign ethnic neutropenia are poorly understood but a recent report has identified genetic deletion of the Duffy antigen receptor for chemokines plays a major role for neutropenia (2). Neutropenias as a consequence of drug treatment can be predictable during cytotoxic courses. However, drug-induced idiosyncratic agranulocytosis (DIAG) is unpredictable and can be fatal. It is defined by ANC under 0.5 x10^9/L (some authors regard an ANC < 0.1 as a limit for DIAG). Neutropenia should occur during drug treatment or within 7 days after exposure to the drug. The incidence of DIAG appears to have changed during the last 30 years and ranges between 0.1-1% of all patients exposed to the drugs most often implicated (3, 4).

This rather stable incidence of DIAG despite drug market changes suggests that factors related to the host are of greater significance for the emergence of this adverse event than the drug itself (3). Recently, late-onset neutropenias (LON) have been reported months after rituximab therapy in patients with lymphoma and autoimmune diseases (5). However, the true incidence and mechanisms are not fully understood. Recent reports imply that genetic factors may play an intriguing role for development of LON, for example, polymorphisms in FCGR3 which may lead to differences in the susceptibility to rituximab treatment. This presentation will give a general background on the nature of acquired neutropenias and will review important recent advances. The main focus will be on DIAG.

O34
THROMBOCYTOPENIA
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Adequate numbers of platelets are required for effective hemostasis. Thrombocytopenia, defined as a platelet count of less than 150 x 10^9 /µL although counts of less than 100 x 10^9 /µL are more likely to be clinically significant, may cause an increased risk of bleeding. Work up of thrombocytopenia focuses on the major mechanisms that may underlie development of thrombocytopenia including decreased bone marrow platelet production, increased peripheral platelet destruction, increased splenic sequestration and dilutional effects. Investigation into the etiology underlying thrombocytopenia to arrive at a final diagnosis requires careful consideration of the clinical history, patient’s age, baseline platelet count and time course for development of thrombocytopenia, as well as any pertinent medical and surgical history (including any bleeding or thrombotic manifestations), family history as well as medication history. In addition, the work-up will require correlation with pertinent laboratory testing, including CBC data, peripheral smear review, and in some cases bone marrow examination and physical examination to identify the presence of bleeding. Based on the CBC and morphologic review, clinical history and physical examination, additional laboratory testing including DIC panels, LDH, direct anti-globulin test (Coombs test), reticulocyte count, total and direct bilirubin or other testing to address specific differential diagnostic considerations can be undertaken. This talk will present the general approach to work up and diagnosis of thrombocytopenia in both adults and children. Examples of diseases representing each of the major mechanisms underlying development of thrombocytopenia will be illustrated with specific disease entities, focusing on non-neoplastic causes of thrombocytopenia. Decreased bone marrow platelet production will be illustrated by examples of congenital thrombocytopenias (with focus on those disorders which are well characterized by mode of inheritance and specific genetic defects). Acquired causes of decreased bone marrow platelet production including toxic or drug exposures and infections will also be addressed. Increased peripheral platelet destruction is a relatively common cause of thrombocytopenia in both children and adults. Immune thrombocytopenia (ITP) as well as immune-mediated drug induced thrombocytopenia will be discussed, and strategies for distinguishing between these entities outlined. Heparin-induced thrombocytopenia (HIT) will be discussed in detail because of its potentially severe bleeding consequences. Non-immune mediated platelet destruction with consumption emphasis of the microangiopathic processes of thrombocytopenic purpura (TTP), hemolytic uremic syndrome (HUS) and disseminated intravascular coagulation (DIC) will be discussed. Testing helpful in the work-up of thrombocytopenia due to increased platelet destruction/utilization and their utility in clinical practice will be presented. The less common mechanisms of platelet sequestration and hemodilution as well as pseudo-thrombocytopenia will be briefly discussed for completeness. Throughout the presentation, the need for integration of clinical and laboratory features as well as the need to recognize the underlying mechanism leading to the development of thrombocytopenia will be emphasized as this is essential for accurate diagnosis and determination of appropriate therapeutic interventions.

O35
LEUKOCYTOSIS - WHEN TO GET WORKED UP?
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An elevated white blood cell count, or leukocytosis, is a common laboratory finding. Appropriate specimen evaluation depends on which lineages are increased and the morphologic findings on peripheral blood smear review in order to guide further testing. The presence of blasts is concerning for acute leukemia and may require bone marrow biopsy. Lymphocytosis may be morphologically divided into polymorphic and monomorphic populations. Polymorphic lymphocytosis is most consistent with a reactive process, while monomorphic populations are concerning for lymphoproliferative neoplasm. The differential can be further narrowed based on morphologic findings. Myeloid leukocytosis can occur in a number of reactive conditions, as well as myeloid malignancies. The types of cells present and morphology can help to guide additional work up. This lecture provides guidance for the appropriate evaluation and further work up of leukocytosis.

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O36
BEST PRACTICE GUIDELINES FOR HEMOGLOBINOPATHY DIAGNOSTICS
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Guidelines offer an overview of recommended strategies and methods for carrier identification and prenatal diagnosis of hemoglobinopathies (1,2). The European Molecular Genetics Quality Network (EMQN) has already defined Best Practice Guidelines (BPG) for hemoglobinopathies diagnostics in 2002 in a meeting organized in Manchester were experts from various countries reached consensus about guidelines made available on the EMQN web-site. After 10 years however methods and technologies have either improved, been replaced or added to the basic hemoglobinopathy laboratory diagnostic practice (3,4,5). Therefore the EMQN organized a meeting in Leiden in 2012 inviting 36 experts from 22 different countries to revise the existing BPG, which after publication will be available on the EMQN web-site. These guidelines focus on best practice in laboratory methods and interpretation of results for carrier identification and prenatal diagnosis. Hematological methods for carrier detection, hemoglobin pattern analysis and methods for DNA analysis will be discussed. Identification of carrier couples and offering them reproductive choices is a way of managing the health burden of the hemoglobinopathies. The hematological laboratory plays a key role in the identification of carriers as hematology and biochemical separation already allows the carrier status rather than DNA analysis. However, the use of DNA analysis is essential to identify complex combinations of hemoglobinopathies in carriers and patients and should be employed when results are unclear or prenatal diagnosis is requested (3). Finally the DNA results should confirm the hematological and biochemical findings.

O37
FETAL HEMOGLOBIN INDUCTION IN SICKLE CELL DISEASE AND THALASSEMIA
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The beneficial effect of fetal hemoglobin (HbF, α2γ2) in β thalassemic and sickle cell disease has been a major driving force for the study of hemoglobin switching and therapeutic approaches for its reactivation. The globin chains that make up the hemoglobin proteins are encoded by genes found in two separate clusters: the α globin genes and their related embryonic ζ globin gene (HBZ) on the tip of the short arm of chromosome 16 (16p3.3), and the β-like globin chains – embryonic ε genes (HBE), the two γ globin genes (HBG1 and HBG2), δ globin gene (HBD) and β gene (HBB) on the short arm of chromosome 11 (Hp15.5). These genes are arranged in the order of their developmental expression pattern The transition from fetal to adult hemoglobin production, referred to as hemoglobin switching, in humans, occurs around the time of birth but it is not Syllabus material ISLH, May 2014 complete in that residual amounts of Hbf continue to be produced throughout adult life. While persistence of Hbf has no consequence in healthy adults, persistent expression of the endogenous γ globin genes has major beneficial effects on the clinical severity of β thalassemia and sickle cell disease (SCD), disorders with substantial morbidity and mortality worldwide. Clinical and twin studies have shown that HbF levels vary considerably in adults and that, this common variation is largely genetically controlled. Our understanding of hemoglobin control, including that of the persistence of HbF synthesis in adults, historically has been based on Mendelian models of inheritance of natural mutants. Indeed, characterization of these mutations associated with hereditary persistence of fetal hemoglobin (HPFH) has led to the concept of gene competition and autonomous gene silencing underlying the fetal to adult Hb switch. Identification of the transcription factors mediating the switch has eluded us until recently. Dawn of the ‘...omics’ era, using genetic, genomic, transcriptomic and proteomic approaches, has provided new insights into the developmental regulation of the fetal and adult globin genes, and identified specific transcription and epigenetic regulators modulating the common HbF variation in adults. These transcription factors (BCL11A, KLFL1, and MYB ) and chromatin modifiers (histone deacetylases, DNA methyltransferases, and nuclear receptors TR2/TR4) are attractive molecular targets for therapeutic modulation of fetal hemoglobin. As always, however, substantial hurdles remain in achieving specificity of effect and adequate therapeutic window. Optimal HbF modulation may well involve a combination of different strategies and molecular targets.

O38
COMPLEX DIAGNOSTICS IN HEMOGLOBINOPATHIES
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Introduction: Management and prevention of the severe forms of hemoglobinopathies imply that the diagnosis of both major syndromes and carrier conditions is properly made. Standard diagnosis workflow after clinical suspicion includes a complete blood count, a protein study and then, in case of abnormal profile, a confirmation by molecular analysis. In some cases, discrepancies between clinical phenotype and protein profile or between protein profile and molecular diagnosis are observed that prompt one to pursue investigations despite a normal profile. Methods: Some hemoglobin variants which cannot be separated from normal Hb A fraction can cause an array of phenotypes such as thalassemia trait or compensatory erythrocytosis, according to their functional properties. In this case, molecular diagnosis will reveal substitutions leading to missense mutation in one or several of the globin chains. Large deletions of regulatory regions which preserve globin genes intact but prevent their expression can be also causative of such phenotypes. In these cases, direct sequencing will give normal results and technologies investigating insertions or deletions such as MLPA or high resolution CGH array will be necessary to characterize the molecular defect. In other cases with pathological phenotypes, the protein profile reflects the clinical condition but does not correlate with the molecular study. These cases are mostly represented by somatic mutations resulting in loss of heterozygosity in patients initially heterozygous for a common globin gene defect. The lost allele can be the one carrying the mutation and in this case, the phenotype of carrier will be attenuated. Conversely, the loss can concern the normal one and in this case, the phenotype will be more severe and similar to the homozygous condition. The loss of heterozygosity can occur either by a deletional mechanism or by recombination leading to uniparental isodisomy (UPD). In both cases, specialized methods such as MLPA or CGH array are not sensitive enough to confirm the mosaicism. The confirmatory diagnosis will be made by FISH in case of deletion and by heterozygosity profile determination through SNP bead arrays analysis when UPD is suspected. Beside those complex diagnostics, a novel aspect of molecular diagnosis is being developed aimed at the study of genetic modifiers of the severity.Disease severity in patients with hemoglobin defects varies greatly. Variant genotyping of genetic modifiers may possibly help in the early prediction of the level of severity the patient will develop later. This prediction tool of severity may have implications for therapeutic decision making.
O39 TTP AND ADAMTS13 TESTING
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Thrombotic thrombocytopenic purpura (TTP) is a rare disorder characterized by microangiopathic hemolytic anemia with schistocytes on the blood smear, thrombocytopenia and a variable degree of organ manifestations due to microvascular thrombosis. Because of mortality rates exceeding 90% if left untreated, therefore, clinical decision making and initiation of treatment (plasma exchange with replacement of fresh frozen plasma) have to be made without delay.

Within the group of clinically diagnosed TTP patients, those with a severe ADAMST13 deficiency represent a special entity as they have an increased risk of relapse, but also of long-term sequelae (neurocognitive deficits, depression, hypertension, etc.). Two forms of severe ADAMST13 deficiency (<5% of the normal) are recognized. The acquired form is the result of circulating autoantibodies to ADAMTS13, which either inhibit ADAMTS13 function or increase ADAMTS13 clearance, while congenital ADAMST13 deficiency is the result of compound heterozygous or homozygous mutations in the ADAMTS13 gene. Nowadays a number of ADAMST13 assays, in house assays and commercial assays are available for ADAMST13 activity and antibody/inhibitor determination. After a pilot in 2013 ECATt offers since 2014 external quality assessment for ADAMST13 activity and inhibitors and towards the end of 2014 the first WHO international standard for plasma ADAMTS13 will become available.

O40 LABORATORY PRACTICALITIES IN TESTING FOR PROTEIN C, PROTEIN S AND ANTITHROMBIN
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Venous thromboembolism is a complex disease known as Thrombophilia. Venous thrombotic disease is caused by both genetic and acquired/environmental risk factors. To date, three major genes have been identified as having significant relevance in Thrombophilia expression: Protein C (PC), Protein S (PS) and Antithrombin (AT). These components are routinely screened in investigations for the cause(s) of VTE. To assess the genetic contributions of PC, PS and AT to Thrombophilia, the only currently practical assessment of their genetic status is by laboratory assay of plasma levels of these factors. Laboratory test methods can be divided into 3 stages: Pre-analytical, Analytical and Post-analytical. Each stage has components and procedures that must be controlled to generate accurate results and the correct interpretation. A brief review of pre-analytical and post-analytical variables affecting test results and interpretations for PC, PS and AT will be presented. References to reviews or documents detailing solving the pre-analytical and post-analytical issues will be provided. However the majority of this lecture will focus on themes concerning, for example, hematopoietic stem cell transplantation.

Results: Conclusions: To conclude, hemoglobinopathies with unusual molecular basis necessitate specialized DNA technologies and experienced interpretation to obtain accurate diagnosis and proper risk assessment.

O41 CURRENT INSIGHT IN THE LABORATORY DIAGNOSIS OF HIT
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Heparin-induced thrombocytopenia (HIT) is an adverse drug reaction and prothrombotic disorder caused by immunization against platelet factor 4 (PF4) after complex formation with heparin or other polyanions. After antibody binding to PF4/heparin complexes, HIT antibodies are capable of intravascular platelet activation by cross-linking Fc gamma receptor IIa (FcγRIIa) on the platelet surface leading to a platelet count decrease and/or thrombosis. In contrast to most other immune-mediated disorders, the currently available laboratory tests for anti-PF4/heparin antibodies show a high sensitivity also for clinically irrelevant antibodies. This makes the diagnosis of HIT challenging and bears the risk to substantially overdiagnose HIT. The strength of the antigen assays for HIT is in ruling out HIT when the test is negative. Functional assays have a higher specificity for clinically relevant antibodies, but they are restricted to specialized laboratories. Currently, a Bayesian approach combining the clinical likelihood estimation for HIT with laboratory tests is the most appropriate approach to diagnose HIT. In this review, we give an overview on currently available diagnostic procedures and discuss their limitations.

O42 ANALYSIS OF HEMATOGONES AND LYMPHOBLASTIC CELLS IN B-ALL USING MULTIPARAMETRIC FLOW CYTOMETRY.
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Introduction: In bone marrow samples of B-lineage acute lymphoblastic leukemia (ALL) analyzed at the end of induction chemotherapy, it is often difficult to differentiate normal (hematogones) and abnormal (blast cells) immature lymphoid cells by microscopic examination but also by immunophenotyping procedures. The detection and quantification of minimal residual disease (MRD) requires a perfect recognition of hematogones and new multiparametric flow cytometry (MFC) procedures (> 8 colors) could be of a great interest to that avail. A new procedure for leukemia immunophenotyping analysis was recently introduced and in particular applied to B-ALL diagnosis and follow-up (GEIL-Groupe d’Etude Immunologique des Leucémies-). Based on 2 ten-color tubes, in some cases it fails to perfectly distinguish between normal and abnormal lymphoid immature cells. It was decided to introduce a new ten-color tube to circumvent this default and first results are shown here. Methods: Thirty normal bone marrow samples obtained from a surgery department or from patients with haematological diseases in remission were compared to 10 samples of B-ALL at diagnosis and follow-up using MFC procedures. The latter included the two ten-color tubes designed by the GEIL group and a new tube (Tube 3) designed as follows: CD44-FITC / CD66c-PE / CD24-ECD / CD49e-PECy7 / CD81-APC / CD10-A700 / CD19-A550 / CD38-PB / CD45-KO. Comparisons between normal and abnormal samples were performed using specific gating on Kaluza®software (Beckman Coulter) with
particular attention to improve the detection of MRD. Results: Using adapted Kaluza™ gating and analysis (in particular, the radar procedure), it was possible to individualize normal and abnormal lymphoid immature cells, with the three panels, but possibly more easily and better with the new combination. A comparison between GEIL tubes and tube 3, with many illustrations will be shown. Moreover, MRD result comparisons were performed between MFC and molecular biology, when available. We focused on sensitivity levels obtained with both methods. Conclusions: Precise distinction between normal and abnormal immature lymphoid cells can now be reached introducing a new panel of monoclonal antibodies and new software analysis. Therefore, a better detection of MRD in B-ALL is suggested by these preliminary results and should be confirmed on larger series of patients.

O43 ASSESSMENT OF PLATELET FUNCTION BY FLOW CYTOMETRY IN FIXED WHOLE BLOOD SAMPLES - EVALUATION OF ITS DIAGNOSTIC POTENTIAL FOR PLATELET FUNCTION DISORDERS

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Platelet function tests are widely used to diagnose inherited platelet function disorders (PFDs). Light transmission aggregometry (LTA), including lumi-aggregometry, is still regarded as the gold standard, however it is time- and labour-intensive and blood samples must be processed within a limited time after venepuncture. Furthermore, a considerable proportion of subjects with suspected PFDs do not show a platelet abnormality on LTA. We have developed an assay for platelet function where fresh whole blood samples are manipulated in a simple way that does not require specialized staff or equipment. The samples are then fixed, which stabilizes them for up to 9 days, and shipped for analysis at a central laboratory. Here we aimed to assess the diagnostic potential of this easy-to-use remote platelet function test (RPFT) as a diagnostic pre-test for suspected PFDs. Methods: RPFT was compared to lumi-aggregometry in participants recruited to the Genotyping and Phenotyping of Platelets study (GAPP, ISRCTN 77951167) from April 2012 to January 2013. Platelet function was assessed in 61 participants who had an history of excessive bleeding (42 index cases and 19 relatives), and in 41 healthy volunteers. For RPFT, whole blood was stimulated with platelet agonists, stabilized with PAMFix (Platelet Solutions Ltd, Nottingham, UK) and returned to the central laboratory for analysis of P-selectin and CD63 by flow cytometry. Results: Overall agreement between lumi-aggregometry and RPFT was good, with diagnosis being concordant in 84% of cases (kappa=0.668, p<0.0001). According to both tests, 29 participants were identified to have a deficiency in platelet function and 22 participants appeared normal. There were 4 participants where lumi-aggregometry revealed a defect but RPFT did not, and 6 participants where RPFT detected an abnormal platelet response that was not identified by lumi-aggregometry. The majority of patients who had an abnormal pattern of response to lumi-aggregometry but not on RPFT had a mild platelet function defect based on dynamic kinetic information from lumi-aggregometry. Conclusions: The study suggests that remote assessment of platelet function in fixed whole blood samples by flow cytometry could provide an easy-to-use pre-test to select which participants with bleeding disorders would benefit from extensive platelet phenotyping. This assay can be performed remotely and requires a small volume of blood, both of which offer significant advantages over lumi-aggregometry. Further development and evaluation of the test are warranted in a wider population of patients with excessive bleeding and could provide an informative screening test for PFDs.

O44 IMMUNOPHENOTYPIC ALTERATIONS OF BONE MARROW MYELOID CELL COMPARTMENTS IN MULTIPLE MYELOMA PATIENTS PREDICT FOR MYELODSYPLASIA-ASSOCIATED CYTGENETIC ALTERATIONS

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Introduction: Improvement in MM treatment is accompanied by a concern for secondary primary malignancies (SPMs). Consequently, it is of major relevance to identify patients at higher risk of developing SPMs by routinely available techniques. Methods: Here, we investigated the presence of MDS-associated immunophenotypic alterations (MDS-PA) in 250 consecutive bone marrow (BM) samples from newly-diagnosed patients with plasma cell neoplasias. Results: Myeloid deviations (aberrant CD56 expression on monocytes, and/or myeloid hypogranulation) were observed in 33/250 patients (13%). These cases were further investigated for the presence of MDS-PA with a broader, MDS-focused monoclonal antibody panel. This confirmed the presence of additional multiple/marked MDS-PA in 20/33 patients (60%). The remaining 13/33 cases only showed mild and/or isolated myeloid phenotypic alterations. Such MDS-PA involved CD34+ cells, maturing neutrophils, mononcytic and/or erythroid BM cells from 90%, 95%, 95%, and 75%, respectively. Similarly, mild/isolated phenotypic alterations were detected in 46%, 69%, 77% and 8% of the same BM cell subsets, respectively. In 28/33 (17 males and 11 females) cases showing either multiple/marked MDS-PA (n=20) or mild/isolated phenotypic alterations (n=8) the presence of clonal markers was specifically investigated in FACs-purified CD34+ cells, maturing neutrophils, monocytes and erythroid cells. In 22 cases (17 males and 5 females), MDS-associated cytogenetic alterations were screened by fluorescence in situ hybridization (FISH); in 10 female patients clonality was investigated by the Human Androgen Receptor Assay (HUMARA). Through these techniques, clonal features of different myeloid cell compartments were detected in 15/28 cases (54%) including 11/20 (55%) patients with multiple/marked MDS-PA and 4/8 (50%) cases with mild/isolated phenotypic alterations. In detail, cytogenetic alterations were present in different BM cell compartments from 7/17 (41%) male patients; 5/7 had multiple MDS-PA in association with del(5q31) (two cases), trisomy 8, del(7q31) and, del(5q31) plus del(7q31), respectively, while the remaining 2/7 cases had isolated/ mild phenotypic alterations in association with nullisomy Y. In turn, none of the 5 female cases showed cytogenetic abnormalities with the FISH-probe panel here used. Conversely, myeloid clonality was confirmed by HUMARA in most female patients (n=8/10; 80%): 6/7 (86%) and 2/3 (67%) depicting multiple/marked MDS-PA and mild/isolated phenotypic alterations, respectively. Additionally, by conventional cytomorphology dysmorphic features were observed
in 7/8 (88%) and 5/6 (83%) cases with multiple/marked MDS-PA and mild/isolated phenotypic alterations, respectively. **Conclusions:** Overall, a significant proportion of MM and MGUS patients with either multiple/marked or even mild/isolated MDS-associated phenotypic alterations at diagnosis also display genetic and/or morphologic evidences of clonal myeloid hematopoesis.

**O45**
**NEXT GENERATION SEQUENCING IN THE DIAGNOSIS OF RED BLOOD CELL MEMBRANE DISORDERS**

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**Introduction:** Hereditary disorders of the red blood cell (RBC) membrane constitute a major cause of hereditary hemolytic anemia. It concerns a heterogeneous group of diseases with highly variable clinical expression. Traditionally RBC membrane disorders are classified according to the morphological appearance on a blood smear. Hereditary spherocytosis (HS) and elliptocytosis (HE) are among the more common abnormalities, whereas hereditary pyropoikilocytosis (HPP) and stomatocytosis (HST) are more rare. HS, HE, and HPP is caused by mutations disrupting the RBC cytoskeleton or its anchoring to the RBC membrane, thereby perturbing the cell’s structure and function. Inheritance of these diseases is in most cases autosomal dominant. Molecular analysis has long been hampered by the large and complex nature of the many different genes involved, and the fact that most mutations are private ones. **Methods:** We implemented an NGS based test in our DNA diagnostics laboratory to sequence seven genes commonly associated with RBC membrane disorders: SPTA1 (α-spectrin), SPTB (β-spectrin), ANK1 (ankyrin), SLC4A1 (Band 3), EPB41 (protein 4.1), EPB42 (protein 4.2), and RHAG (Rhesus-associated glycoprotein). 33 patients with defined membranopathies were selected. After enrichment of genomic DNA, using a custom Agilent SureSelectXT probe kit, protein coding and flanking intronic sequences were determined using a SOLID™-5500XL system. Variants were identified using an ‘in house’ developed NGS mapping and calling pipeline, and the Cartagenia BENCHlab NGS module was used for filtering and prioritization of possible pathogenic variants. Detected mutations were confirmed by conventional Sanger sequencing. **Results:** Probable causative mutations were identified in 27/33 (85%) of patients studied, representing 29 unique mutations. Most mutations were located in SPTA1 and ANK1. Somewhat surprisingly, no mutations were detected in the gene encoding band 3 (SLC4A1), a major cause of HS. As was expected, most of the detected mutations were novel (26/29) and private. **Conclusions:** The here presented approach represents a feasible and reliable diagnostic method to detect mutations in patients affected by disorders of the RBC membrane. Establishing the molecular diagnosis will be particularly important in young children with congenital anaemia, transfusion-dependent patients, and in families with variable clinical expression or complex inheritance patterns. In addition, understanding the molecular mechanisms involved in disturbed RBC membrane function will contribute to a better understanding of normal RBC physiology.
O47 LABORATORY HAEMATOLOGY IN SUB-SAHARAN AFRICA: INSIGHTS FROM SOUTH AFRICA

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South Africa (SA) has the highest burden of Human Immune Deficiency Virus (HIV) infection in the world with 5.6 million South Africans living with HIV in 2012. The number of people in SA receiving antiretroviral (ARV) therapy reached 2.15 million in 2012 which is the largest ARV rollout in the world. Tuberculosis (TB) and HIV are closely linked with TB being the leading cause of death for those HIV infected in SA. In 2011 the prevalence of HIV infection amongst antenatal clinic attendees was 29.5%, which has shown a plateau since 2005.

The National Health Laboratory Service (NHLS) is the public health laboratory service which provides a diagnostic pathology service to the vast majority (84%) of South Africans who do not have private medical insurance, and comprises 268 laboratories across South Africa. The HIV epidemic has placed high demands on NHLS haematology laboratories, with CD4 cell enumeration and full blood counts forming a central role in HIV patient monitoring. HIV and TB testing in SA accounts for 30% to 40% of all public sector laboratory expenditure, and in 2012 the NHLS performed ~ 4 million CD4 counts as part of HIV monitoring. CD4 testing is performed using two colour, single platform, panleucogucate (PLG) technology which has demonstrated accuracy in international quality assurance programs, and accommodated high workload which in some laboratories reaches 3000 tests/month. Point of care testing including CD4 counts, haemoglobin levels, and geneexpert testing for TB is being evaluated for widespread implementation in ~ 3500 HIV clinics across the country.

Although many rural laboratories have only basic infrastructure, test availability and transport access, academic tertiary centres can offer sophisticated haematology diagnostics including 8 colour flow cytometry, cytogenetics, FISH analysis and molecular testing.

The high incidence of HIV in SA results in haematological complications being a common clinical problem. The more serious complications include the high grade B cell lymphomas, Hodgkin lymphoma and thrombotic thrombocytopenic purpura. The diagnostic and clinical approach to these complications will be briefly discussed.

O48 HEMATOLOGY IN TANZANIA

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Introduction: Longstanding ISLH member, Dr Monica Gallivan, passed away in 2013. Her passion was to improve the diagnosis and treatment of patients with hemoglobinopathies. She visited Tanzania where she trained me to interpret hemoglobin studies including electrophoresis and HPLC. This resulted in my sponsorship by the American Society of Hematology (ASH) and subsequent improvements in hematology services and training in Tanzania. Through ASH, I was able to train in haemoglobinopathy diagnosis at Quest Diagnostics Nichols Institute Chantilly, Virginia-USA, under the mentorship of Dr Gallivan. I also learnt about the optimal clinical management of sickle cell disease (SCD) patients during a clinical attachment at Howard University (Washington, DC) under the supervision of Dr Patricia O’Neal. As a result of this training, I was able to conduct a Tanzanian study in 2009 on newborn screening (NBS) for haemoglobinopathies as part of my masters (dissertation) program. I screened over 2,000 births and established the feasibility of NBS in Tanzania. This work was funded by a centenary scholarship award I received from the Royal Society of Tropical Medicine and Hygiene (London, UK) in 2009.

Methods: Muhimbili National Hospital (MNH) serves as the national referral hospital, for hematology healthcare in Tanzania. MNH now has six hematologists, supported by related specialists and affiliated healthcare professionals providing clinical care to hematology patients. In 2004, the Muhimbili sickle cell cohort was established at MNH, by Dr Julie Makani as part of her PhD project. This project now includes over 3500 patients with HbSS and has led to a detailed description of the clinical spectrum of the disease; including the mortality and morbidity due to malaria, bacterial infections and stroke. The challenges we have in Tanzania include a lack of trained personnel, poor funding and poor diagnostic facilities, especially in rural areas. We do not have enough qualified hematologists, technologists, researchers and specialized nurses to adequately cope with the large population of Tanzanians (45M). However within the last 10 years, our hematology division has developed and grown and we now have 6 qualified hematologists (versus two 10 years ago). We have also managed to get our Clinical Hematology Unit recognized at MNH within the Department of Internal Medicine. I am the Head of Clinical Hematology and we have three allocated nurses and three medical doctors employed by the hospital working in clinical hematology. Our hematology patient load is 100 – 120 out patients/week and 30 to 50 inpatients per week. We diagnose hematological malignancies on morphology and prescribe chemotherapy for chronic hematological malignancies. For the acute leukemia and lymphoma patients, who need combined intravenous chemotherapy; we refer them to a specialized cancer hospital for treatment after making diagnosis. We have also built our research and training capacity in hematology and now offer a number of courses including a PhD program (currently with 2 students), MMed, MSc courses Bachelor of Medical Laboratory Sciences (BMLS-currently with 5 students), and an Advanced Diploma in Medical Laboratory Sciences (ADMLS) We are fortunate to have been included in the Health Volunteer Overseas (HVO) program in collaboration with ASH, whereby different volunteers come to MNH and MUHAS to teach. Four volunteers have visited thus far. We have succeeded in establishing the Sickle Cell Foundation of Tanzania (SCFT) in June 2010; the Hemophilia Society of Tanzania (HST) in April 2009 and World Federation of Hemophilia (WFH) Hemophilia Treatment Centre Twinning Program in 2013. We have had a number of local television and radio programs, as well as cycling and walking campaigns to raise awareness of different hematological disorders to Tanzanian and East African people.

Results: Conclusions: Our future direction aims are to establish a centre of excellence for sickle cell management at MNH, and to develop the Hematology Department and Blood Transfusion Institute. We are preparing to start a newborn screening program for hemoglobinopathies in collaboration with Department For International Development-UK (DFID), the Wellcome Trust and the Tanzanian Ministry of Health and Social welfare; using the study I performed in 2010 as a pilot study. Finally, we would like to build and develop a Tanzanian Hematology Association.
O49
INTRODUCTION
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Introduction: The International Council for Standardization in Haematology (ICSH) is a not-for-profit organisation that aims to achieve reliable and reproducible results in laboratory analysis in the field of diagnostic haematology. ICSH coordinates Working Panels of experts, works in cooperation with international organization, deliberates on issues of standardization and develops international standardization materials and guidelines. Activities of the ICSH do cover all the aspects of the laboratory hematology. An overview of recently published and on going projects will be summarized in the following paragraphs. Over the past few years the following ICSH documents have been published: i) ICHS guidelines for the standardization of bone marrow specimens and reports, ii) ICSH review of the measurement of the erythrocyte sedimentation rate, iii) CSH guidelines for the standardization of bone marrow specimens and reports, iv) Recommendations for identification, diagnostic value, and quantitation of schistocytes, v) Toward a reference method for leucocyte differential counts in blood: comparison of three flow cytometric candidate methods, vi) Recommendations for the measurement of Haemoglobin F, vii) Recommendations for the measurement of Haemoglobin A2, viii) Practice guidelines on validation of cell-based fluorescence assay, in association with ICCS, published in five different parts: a. rationale and aims b. preanalytical issues c. analytical issues d. postanalytic considerations e. performance criteria. In agreement with its original mission statement, ICHS submits most completed Guidelines and Recommendations as ICSH publications to the ISLH journal, International Journal of Laboratory Hematology. All documents undergo the appropriate review process of the Journal. The first four of the above mentioned published documents have been recorded among the top 10 most downloaded articles published by the International Journal of Laboratory Hematology in 2012. This confirms and underlines how ICHS continues to play an important role in diagnostic laboratory haematology worldwide. Current ICHS Working Panels (WPs) are focused on different aspects of diagnosis and the work flow of the hematology laboratory. Validation of innovative technologies and methods remains a main goal of the Council, and new documents are being discussed, prepared and finalized. Two of them have recently been accepted for publication: i) ICHS Guidelines for the Validation of Automated Analyzers for Body Fluids (Dr Gini Bourner as chair). ii) Validation of Automated Haematology Analyzers (Dr Carol Briggs and Dr Naomi Culp as chairs). Dr Brent Wood chairs the WP aimed to finalize ICSH Guideline for the Extended Differential Reference Method. As for Red Blood Cell analysis, Dr May-Jean King (UK) and Dr Adrian Stephens (UK) chair WPs for Guidelines for the Laboratory Diagnosis of Non-immune Hereditary Red Cell Membrane Disorders and for the Assessment of Automated Equipment for the Measurement of HGB A2, respectively: as for HGB A2, a preliminary assessment has shown a large variation between existing equipment. Three separate WPs are working in areas related to blood and bone marrow cell morphology: - Standardization of Peripheral Blood Film Review and Reporting by Physicians (Dr Maria Proytcheva as the chair): the project has already carried out an international survey, which showed a large variation in practices for review and reporting, confirming that a consensus on interpretation and reporting is important for patient care and guidelines will be developed; - Bone Marrow Immunohistochemistry (Dr Emina Torlakovic and Dr Anna Porwit as chairs); - Cell Morphology Grading (Dr Stefanie McFadden and Dr Gina Zini as chairs). In the field of the diagnosis of hematologic neoplasms, Dr Wolfgang Kern and Dr Marie C. Béné do chair a WP aimed to develop ICSH Guidelines for Flow Cytometric Evaluation of AML and MDS. The operative project will lead to an original, algorithm-based diagnostic approach for MDS, AML and will also deal with sampling handling, antigens, data acquisition, analysis and reporting, quality control and personnel training. A great interest is focused on the standardization of the outputs of results from the hematology laboratory too. Two WPs are working to produce ICSH Guidelines for: - Communication of Critical Results (Dr Tee Beng Keng as chair); - Standardisation of Haematology Reporting Units and Reference Ranges (Michelle Brereton as chair): preliminary work has shown that few countries have unified systems of reporting and that no single parameter has universal acceptance, so that there is a strong need of standardization for common computer systems. At the web site http://icsh.org all the information and published documents and guidelines are freely available. Hopefully, laboratory hematology experts will continue to use ICSH documents and also to actively participate in the process of their production. Membership to ICHS will continue to be welcome to any corporation or individual with an interest in standardization issues in laboratory haematology.

O50
FLOW CYTOMETRIC EVALUATION OF MDS/AML
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Introduction: Immunophenotyping by multiparameter flow cytometry (MFC) is widely used in both research and routine diagnostic laboratories all over the world and serves most important results to research projects as well as assignment of patients to specific diagnoses and conditions relevant for their therapy. Modern technologies provide the flow cytometrist with significantly improved capabilities of applied assays. Given this value of MFC as applied today the assurance of quality remains on of the most important topics particularly in the clinical setting. A variety of guidelines for different applications have been published to help the flow cytometrist to provide diagnostic information most accurately. ICSH guidelines have been lacking so far for the flow cytometric evaluation of patients with suspected acute myeloid leukemia and myelodysplastic syndromes and are in preparation at present. MFC is a critical part of the integrated diagnostic work-up of patients with suspected hematologic malignancies, which also includes morphological/numeric identification of hematopoietic abnormalities as well as cytogenetic and molecular investigations. Depending on the suspected disease and on information available from clinical and basic laboratory assessments, various techniques are applied for differential diagnosis. A correct diagnosis is indeed essential to select the most effective clinical management. The latest “WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues” [1] issued in 2008 is the current gold standard for diagnosing and subclassifying hematologic malignancies, within the framework of a multi-modal diagnostic approach. Besides this classification, a variety of national and international consensus and guideline documents have been published, focusing on distinct diseases or disease groups as well as on the application of specific diagnostic techniques [2-7]. These documents provide significant help for both the laboratory performing diagnostic procedures and the treating physician who relies on the laboratory report to manage patients. The “ICSH guideline for flow cytometric evaluation of patients with suspected acute myeloid leukemia and myelodysplastic syndromes” will aim at providing an up-to-date tool for both laboratory professionals and
treating physicians. “Suspected” acute myeloid leukemia (AML) or myelodysplastic syndrome (MDS) are here considered as recently identified cytopenia, rather than already assessed AML or MDS per se. Thus, this guideline provides information on how to apply immunophenotyping at specified check points in the diagnostic work-up pathway and how to derive decisions on further diagnostic/therapeutic steps from available results. In patients with suspected MDS and/or AML, the two major findings that may lead to the application of immunophenotyping are the presence of peripheral blood (PB) cytopenias, and/or the suspicion of blasts detected by routine blood count testing or PB smear examination. Thus, the first task of immunophenotyping in this setting is to search for an increase in immature hematopoietic progenitors with blast morphology. The second task is to evaluate the normality of myeloid, erythroid, and potentially platelet maturation. Accordingly, the adequacy of the samples’ source as well as the definition of abnormal progenitors has to be considered. Regarding the definition of abnormal progenitors, one has to consider that the blast cell type defined by specific cytomorphologic features is not necessarily reflected by a predictable immunophenotypic profile. Immunophenotyping should therefore rather refer to “progenitor cells” or, in case of AML and MDS, to “myeloid progenitor cells”. The basis for the definition of myeloid progenitor cells (MPCs) is their characteristic CD45intdim+/side scatterlow (SSClow) pattern and the presence of such myeloid markers as CD13, CD33 and CD117. In addition, the progenitor marker CD34 and HLA-DR are typically expressed on such cells. However, deviations from these typical findings may be present in both AML and MDS. Pathologic findings in MPCs include an increased percentage of above 5% as one criterion for the diagnosis of MDS and above 20% for the diagnosis of AML. However, these criteria are based on the morphologic observation of selected microscopic fields of BM smears initially obtained from the first drops of a BM aspiration, and not necessarily reflected in further collected and usually hemodiluted samples. In addition, MPCs may display an aberrant immunophenotype, i.e. cross-lineage expression of lymphoid antigens, expression of mature myeloid antigens and/or lack of normally expressed myeloid antigens. Such aberrant antigen expression can be used, even in the absence of an increased percentage of myeloid progenitor cells, as a further criterion for the diagnosis of MDS or, in case of previously treated AML, as target for monitoring minimal residual disease (MRD). Further details will be given in chapter 3 of the guideline. Based on the identification of less than 20% MPCs in the BM, the decision is made to further analyze the sample for aberrant antigen expression in additional cell lineages, i.e. granulocytes, monocytes, erythroid cells, as well as in the B-lymphoid progenitor cells compartment. The panel of antigens to be assessed must focus on the most important and most frequent MDS-related findings and take into account some specific expression patterns. Whenever 20% or more blasts are identified morphologically on BM smears, a diagnostic approach different from that of suspected cases of MDS should be applied. In such cases, one should focus on characterizing the nature of the MPCs and determine their lineage and degree of immaturity. In addition, attempts to identify specific markers related to clinically relevant genetically defined subentities of AML should be made, as well as to identify markers useful for MRD monitoring. Regarding the subclassification of AML, there is a significant, almost not complete, relationship between characteristic immunophenotypes and specific genetic alterations. These must be recognized to initiate specific, more cost-effective diagnostic approaches for respective alterations, i.e. fluorescence in situ hybridization and/or molecular genetic methods, if no extensive first line molecular screening is performed. Further aspects of the guidelines will deal with the differential diagnosis of AML and MDS, quality control and reporting of results. With this comprehensive review of well-reported requirements and guidelines for proper diagnosis of AML and MDS in a context of cytopenia(s) or suspect cells (blasts), by correct usage of MFC, ICSH aims to provide a useful tool for laboratories and practitioners involved in this field.

O51

SUMMARY OF HAEMOGLOBIN, HBA2 AND OTHER HAEMOGLOBINOPATHY PROJECTS

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Introduction: The International Council for Standardization in Haematology (ICSH) in conjunction with Eurotrol have released a new lot of hemoglobin cyanide (HiCN(Fe), HiCN) standard. This is regularly validated by an international group of laboratories to ensure that it is stable and that the assigned value is correct [1]. It is important that this material is kept available to ensure that haemoglobin measurements throughout the world remain consistent. The material is available from Eurotrol (www.eurotrol.com) in packages of five ampoules. Haemoglobin A2 (HbA2) only comprises a small proportion of the total haemoglobin but it is a very valuable marker for the presence of beta thalassaemia trait. The normal level of HbA2 is between 2.0 and 3.5% of the total haemoglobin. In the presence of beta thalassaemia trait the level is raised, usually to a level between 3.8 and 6.0% of the total haemoglobin. For these reasons it is extremely important that the measurement of HbA2 is both accurate and precise. Although the ICSH has recently published a review of methods available for the measurement of HbA2 [2]. It has now become evident from examination of EQA [3] and other Proficiency testing results that different instruments from different manufacturers are giving different results for HbA2 and that this may have significant clinical implications. A group has therefore been tasked with producing recommendations as to how instrument manufacturers and laboratories should assess their equipment before using it to analyse patient samples and it is hoped that this will soon be available for publication. The latest ICSH red cell project is to review the laboratory diagnosis of Hereditary Stomatocytosis and the inherited disorders of the red cell cytoskeleton [4]. Hereditary Stomatocytoses are associated with abnormal monovalent cation permeability resulting in altered red cell volume. Patients with stomatocytosis often present a milder haemolysis when compared with those having a defective red cell cytoskeleton, which is located on the cytoplasmic side of the lipid bilayer. The latter group includes Hereditary Spherocytosis (HS), Hereditary Elliptocytosis (HE), and Hereditary Pyropoikilocytosis (HPP). The project team aims to assess the limitations of the screening tests currently in use for hereditary spherocytosis; namely, the Osmotic Fragility Test, the Acid Glycerol Lysis Time Test, the Eosin-5’-Maleimide (EMA) Binding test, and ektacytometry [5]. The relevance of further investigations (e.g., SDS-polyacrylamide gel electrophoresis and molecular genetic testing) in the management of patients with membranopathy will be discussed in brief. The diagnostic potential of new technology in the diagnosis of membrane defects will also be considered.
INTERNATIONAL STANDARDIZATION OF HAEMATOLOGY REPORTING UNITS – IS IT EVER ACHIEVABLE?

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The aim of this proposal is to consider the opportunities to introduce international standardization for routine laboratory haematology units of measurement and nomenclature, focusing on the blood cell count. There is an ever increasing need to standardize, where possible, in haematology. [1][2]. For any one patient, the pathology services used may be sourced from a variety of different providers [3]. The reasons for using various providers may be financial, depend on location or transport facilities, or by the need to access specialist pathology services not provided locally. Patients may move between pathology providers, having treatments for different conditions at different healthcare units, but may still require one clinical pathology record. Patients access pathology services not just from various providers within a country but from different countries.

The expansion of information technology to transfer data nationally and internationally supports the need for standardization of reporting where possible [4][5]. Electronic data transfer facilitates the increase in international business with samples being transported across international boundaries for testing. In some countries government led initiatives aim to increase the use of the electronic patient record, collating all data for a single patient to a single data resource.

Equally the cost of pathology in healthcare is a major consideration, with mergers and multi-laboratory partnerships formed across healthcare services, thus providers of large scale pathology require high levels of standardization. Furthermore the last 10 years have witnessed a blossoming of Point of Care Testing (Near Patient Testing) technology, enabling patients to have routine blood tests in less regulated locations (non-healthcare environments), some without clinical infrastructure or on-site expertise. Considering patient safety, it is the duty of the pathologist to remove confusion over laboratory reporting and eliminate differences, where no valid clinical reason for the difference exists. By providing professional guidance to users of our services we can send a clear clinical message that is transferable between pathology providers. ICSH agreed to a pilot exercise to investigate the level of standardization for the blood cell count at a national level. Members from 11 nations agreed to submit information and complete a questionnaire requiring them to list the units of measurement for the test parameters of a routine blood cell count and comment on whether their country had nationally standardized units.

From the pilot data, only Japan and the Netherlands stated the use of formally agreed units of measurement, at a national level, for all parameters of the blood cell count. Some members stated their countries followed “recommended” units from named clinical professional bodies but with no national enforcement of the recommendations. Australia stated good consensus nationally. On the question of national publications, apart from the Netherlands, which use mmol/L for Haemoglobin, there appeared few national standards, overall journals require authors to use Systeme International d’Unites (SI unit), however there are accepted variations, especially in regards to Haemoglobin where ‘g/L’ and g/dL are both used, depending on the country. The litre (L) has been internationally accepted as a measure of volume at the Conference Generale des Poids et Mesures.

These data are preliminary but suggest that there is wide variation in reporting units for the routine cell blood count. There are many legitimate reasons why laboratories have developed different ways of reporting the basic blood cell count profile. Few countries reported a system offering national guidance or regulation, however where a national unified system has been adopted those countries may have little incentive to change.

The growth of electronic reporting systems and the increase in global healthcare providers (companies offering healthcare and international instrument manufacturers) are driving the need for harmonisation and standardization, at least on a national basis and where clinically appropriate. As there is variation in reporting, guidance is needed, however this requires input and resources from the national professional organizations, areas for consideration:

- Reporting units for cell counts.
- Reporting of white cell differentials, consideration of absolute values and percentage.
- Reporting of Haemoglobin, where several different options are available nationally consider whether a recommendation to standardize is possible.

Advances in technology and competition between analyser manufacturers have reduced the variation in test results obtained from a peripheral blood sample analysed via a routine blood cell count analyser. This is particularly true for haemoglobin. Differences do exist but where these differences are not clinically significant we should consider a pragmatic approach.

Ultimately test results must be meaningful to the user and they must also be easy to interpret. If results are reported in different formats there is both a clinical risk that they will be misinterpreted and a danger that abnormal results may be simply missed. This pilot has highlighted the areas of nomenclature and units of measurement where standardization is feasible, however the challenge is immense and any recommendations would need approval by the full ICSH Council.

Committee members contributing data to this exercise:

Dr Katherine Marsden – Australia RCPA
Dr JM Jou – Spain
Dr Jin-Yeong Han – Korea
Prof Ming Ting Peng – China NCCL
Prof Yohko Kawai – Japan
Prof Keith Hyde – United Kingdom NEQAS.
Dr Thomas Nebe - Germany
Richard McCafferty – Republic of Ireland
Dr Jean-Francois Lesesve – France
Dr Ton Ermens – the Netherlands
Dr Joan Etzell – USA CAP.

UPDATING THE CAUSES OF PLATELET DISORDERS AND FUNCTIONAL CONSEQUENCES

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Platelets derive from megakaryocytes in the bone marrow that deliver all the molecular machinery the platelet needs to mediate the different processes of primary hemostasis including adhesion,
OS4
HOW SHOULD WE TEST FOR PLATELET DISORDERS: INSIGHTS FROM INVESTIGATIONS OF SUBJECTS WITH BLEEDING PROBLEMS
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Heritable platelet function disorders (HPFD) are a heterogeneous group of bleeding disorders with diverse clinical and laboratory characteristics. In contrast to the severe phenotype disorders Glanzmann thrombasthenia and Bernard Soulier syndrome, most non-severe HPFD are incompletely characterised. This is a consequence of the poor standardisation of diagnostic tests and of the lack of consensus about diagnostic criteria for the different sub-groups of non-severe HPFD. Distinguishing patients who have a non-severe HPFD from those who do not is an essential first step in diagnosis which may be aided by bleeding assessment tools and screening tests such as the Platelet Function Analyzer-100. However, high diagnostic accuracy can only be achieved with both light transmission aggregation (LTA) and secretion tests, for which streamlined agonist panels may be of similar utility to extended panels. Standardisation of the methodology of these tests and quality assurance are essential for robust diagnosis. Identification of which platelet pathway is defective in patients with non-severe HPFD is also usually possible with LTA and secretion tests. This strategy also sometimes enables exact diagnosis by implicating a single candidate protein and gene. Next generation sequencing may offer a rapid approach to diagnosis of non-severe HPFD, although rigorous strategies must be adopted to distinguish causative gene defects from bystander variations.

OS5
UPDATE ON VON WILLEBRAND FACTOR TESTING: COMPARISON OF NEW AND OLD ACTIVITY ASSAYS FOR DIAGNOSING AND MONITORING VON WILLEBRAND DISEASE
Catherine Hayward
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Diagnostic tests for von Willebrand disease (VWD) are important for the assessment of VWD, which is a commonly encountered bleeding disorder worldwide. Technical innovations have been applied to improve the precision and lower limit of detection of von Willebrand factor (VWF) assays, including the ristocetin cofactor activity assay (VWF:RCo) that uses the antibiotic ristocetin to induce plasma VWF binding to glycoprotein (GP) IbIXV on target platelets. VWF-collagen binding assays, depending on the type of collagen used, can improve the detection of forms of VWD with high molecular weight VWF multimer loss, although the best method is debatable. A number of innovations have been applied to VWF:RCo (which is commonly performed on an aggregometer), including replacing the target platelets with immobilized GPIbα, and quantification by an enzyme-linked immunosorbent assay (ELISA), immunoturbidometric or chemiluminescent endpoint. Some common polymorphisms in the VWF gene that do not cause bleeding are associated with falsely low VWF activity by ristocetin-dependent methods. To overcome the need for ristocetin, some new VWF activity assays use gain-of-function GPIbα mutants that bind VWF without the need for ristocetin, with an improved precision and lower limit of detection than measuring VWF:RCo by aggregometry. ELISA of VWF binding to mutated GPIbα shows promise as a method to identify gain-of-function defects from type 2B VWD. The performance characteristics of many new VWF activity assays suggest that the detection of VWD, and monitoring of VWD therapy, by clinical laboratories could be improved through adopting newer generation VWF assays.

OS6
HOW LYMPHOMA CAN AVOID DETECTION: PITFALLS IN FLOW CYTOMETRIC IMMUNOPHENOTYPING OF LYMPH NODE BIOPSIES
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Flow cytometric immunophenotyping (IPT) plays an increasing role in the characterization of hematologic malignancies in bone marrow and blood samples. However, the role of IPT in the characterization of lymphoma in lymph nodes is still under discussion. The major contribution of IPT to lymph node diagnostics is to discriminate reactive hyperplasia from B-cell non-Hodgkin lymphoma (B-NHL), especially in fine needle aspirates and core biopsies. Also a correct classification of B-NHL is frequently possible by using a combination of these techniques, even when the amount of cells or tissue is limited. But some notorious problems persist and important lessons can be learned from applying routine IPT on excised lymph nodes, which allows comparison with histology and additional techniques as clonality studies and cytogenetics. In this presentation pitfalls in the daily practice of lymph node diagnostics will be discussed focussing on general characteristics, such as lymphoid scatter patterns and lineage specific antigens, that are used to define lymphoid populations. The absence of these characteristics on proliferating lymphoid cells can potentially lead to an incorrect diagnosis, but, on the other hand, can also be helpful in the discrimination between normal and aberrant cell populations, e.g. lymphoma cells that lack important lineage characteristics.
Sporadic examples of reactive lymphoid proliferations with similar phenotypes are also discussed, illustrating the need for the correlation of IPT with morphology, cytohistochemistry and clinical features.

O57
ANTI-PHOSPHOLIPID ANTIBODY TESTING AND STANDARDISATION
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The antiphospholipid syndrome (APS) diagnosis relies predominantly on laboratory results as the incidence of clinical symptoms (thrombosis and pregnancy complications) is high and often determined by other underlying factors. Assays that measure antiphospholipid antibodies (aPL) must be sufficiently sensitive and specific to classify patients correctly as having APS since over- as well as mis-diagnosis has severe clinical implications regarding treatment. However, the laboratory diagnosis is complicated by the lack of golden standards and all assays show methodological shortcomings. The laboratory criteria (lupus anticoagulants (LAC), and/or anti-cardiolipin (aCL) antibodies and/or β2-glycoprotein I antibodies (aβ2GPI)) that defines patients with APS) were set in the Sydney criteria published in 2006, and lead to a substantial improvement of the APS diagnosis. Nevertheless, a number of questions on the laboratory diagnosis of APS remain unresolved. In addition, in 2009 specifications for LAC detection were provided by the Scientific Standardisation Subcommittee (SSC) of the International Society of Thrombosis and Haemostasis (ISTH). Recommendations for aCL and aβ2GPI assays intended to ameliorate the performance of these solid-phase assays. I will focus on the aPL detected by solid phase assays; mostly enzyme-linked immunosorbent assays (ELISA), although newer automated platforms have been introduced into the market. For aCL and aβ2GPI testing, several factors contribute to variability in pre-, post- and analytical conditions, many factors related to the assay itself and its calibration. I will focus on the efforts and achievements in standardization, and on the weaknesses and strengths of the current available laboratory methods. Despite efforts so far, standardization is not reached yet, but progress have been made. On-going efforts to reduce the inter-laboratory/inter-assay variations remain important, even an absolute standardization cannot be feasibly achieved. We have to take into account the methodological shortcomings and be aware of the performance characteristics of the means we have available.

O58
GUIDELINES FOR LUPUS ANTICOAGULANT TESTING
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Commonalities and contrasts in ISTH, BCSH and CLSI guidelines for lupus anticoagulant detection
LA detection continues to be problematic due to antibody heterogeneity, reagent and analytical variation and the lack of a gold standard or reference preparation. Thus, LA are detected by inference based on their inhibitory behaviour in phospholipid-dependent coagulation assays and their mixing studies that can be reduced or abolished upon performance of the same assay rendered less responsive to LA, commonly achieved by increasing phospholipid concentration. The International Society on Thrombosis & Haemostasis (ISTH) published an update of its 1995 guideline in late 2009, and the British Committee for Standards in Haematology (BCSH) updated their 2000 guideline in early 2012. The Clinical and Laboratory Standards Institute (CLSI), based in the USA but comprising an international panel, published its first LA guideline in early 2014. Although incomplete standardisation has resulted in incomplete agreement between contemporaneous expert panels, we are nonetheless closer to engendering common practices.

Pre-analytical issues
Each guideline recommends double centrifugation of blood collected into 3.2% tri-sodium citrate to ensure generation of platelet poor plasma with a platelet count of <10 x 109/L. Filtration is not recommended due to loss of some clotting factors and potential to generate microparticles. Standard routine coagulation screening tests are valuable to exclude undiagnosed coagulopathies and undisclosed anticoagulant therapy. CLSI further recommends use of a LA-unresponsive APTT reagent to reduce serendipitous finding of LAs in asymptomatic patients and permit interpretation of LA assays unencumbered by the possibility of a co-existing abnormality.

Lupus anticoagulant assays
ISTH recommends employing only dRVVT and a suitable APTT for LA detection. Numerous other assays that have been described over the years, such as KCT, dPT and other snake venom-based assays are not recommended. Reasons given include reproducibility, reagent variability and standardisation issues. CLSI recommends that dRVVT and LA-responsive APTT are performed as first-line screening tests whilst use of other assays is not excluded providing they each employ different pathways in their design. BCSH specifically recommends dRVVT and suggests a suitable APTT would normally be the second assay but others are not excluded. BCSH intentionally describes laboratory criteria as ‘classical findings’: (i) prolongation of a phospholipid-dependent clotting assay (ii) demonstration of inhibition in mixing studies (iii) demonstration of phospholipid-dependence. This is because recognition is given to the potential to dilute LAs in 1:1 mixing studies, thus reducing sensitivity, and that screen & confirm results from undiluted plasma alone can reveal a LA when no other causes of elevated clotting times are present. This parallels the ISTH suggestion that performing screen and confirm, so-called integrated testing, on every patient does not, in principle, require mixing tests. CLSI goes one step further and re-prioritises the testing sequence to screen, confirm and then the mix only if required. Using the mixing test result as a decision point to complete the LA test medley risks the reporting of false negative interpretations when a fundamental limitation of mixing test design is masking a genuine antibody. However, mixing tests increase specificity and diagnostic accuracy when there are co-existing abnormalities, the confirm test on undiluted plasma does not return to the reference range or the co-factor effect is present, and continue to have a place in the analytical armoury.

All three guidelines recommend converting clotting times to ratios to mitigate for daily analytical variation. ISTH and BCSH indicate this can be done using results from normal pooled plasma, whilst CLSI recommends use of the reference interval mean.

Cut-offs
All three guidelines indicate that cut-offs must be locally derived based on specific reagent/analyser pairings. ISTH recommend applying the 99th percentile for determining cut-offs, yet obtaining sufficient donors to do so accurately is beyond the reach of most diagnostic departments. CLSI maintains that 97.5th percentile can
be adopted and points readers to its own reference range guideline for further detail. BCSH considers inaccuracy in relation to sample numbers and suggests that previously established cut-offs can be validated with smaller numbers. It should be recognised that increasing cut-offs to the 99th percentile improves specificity but inevitably reduces sensitivity. Furthermore, whilst use of 99th percentile will reduce frequency of false positive screening tests it will also increase that of false negatives. CLSI indicates that any elevated screening test will receive a confirm test that will be similarly elevated if not due to a LA, so ultimately, a false positive interpretation will not ensue. All three guidelines describe calculations for assessing phospholipid dependence and mixing test interpretation and ostensibly concur.

LA testing during anticoagulant therapy
BCSH specifically states that the majority of patients on VKA therapy can receive LA testing upon cessation of the treatment to avoid analytical complications. Despite this, it is not uncommon for laboratories to receive requests to test on such patients and guidance is given in all three documents. The utility of LA assays performed on undiluted plasma is disputed. Undertaking screen and confirm assays on 1:1 mixtures of test and control plasma can reveal a LA if it is sufficiently potent to overcome the dilution and the confirm step will reveal phospholipid-dependence. Unlike ISTH, no restrictions are suggested based on INR. Negative testing does not exclude a LA due to the dilution effect. Both BCSH and CLSI accept that TSVT screening, with either ecarin time or platelet neutralisation procedure as confirmatory tests, can be a useful adjunct.

BCSH discourages testing on patients receiving unfractionated heparin and ISTH indicates that LA screening is not possible if plasma is unclottable or the heparin level exceeds reagent-integral neutraliser capacity. CLSI gives examples where heparin neutralisers in DRVVT reagents are successful in revealing a LA and when they are not. Only CLSI covers the issue of effects of new oral anticoagulants, most of which interfere to some degree with LA testing. TSVT and ecarin time are, however, inevitably unaffected by direct FXa inhibitors.

Reporting
All three guidelines concur that reports should include an expert interpretation of the potentially complex set of results.

O59 DETECTION OF PLASMA CONTACT SYSTEM ACTIVATION IN THROMBOTIC DISEASE
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The contact system is a volatile and versatile enzyme system in blood plasma that responds to the presence of non-physiological surface materials by spontaneous generation of enzymatic activity. In subsequent steps, it can trigger blood coagulation and is responsible for the generation of the inflammatory peptide bradykinin. The physiological role of the contact system is presently unknown, but it is commonly used to trigger coagulation in a diagnostic setting. During experimental studies in vivo, contact system activation has been implicated in several pathological conditions, ranging from thrombosis to anaphylactic shock and (hereditary) angioedema. However, the contributions of the contact system to human pathological states is less well established. This presentation will first describe the molecular mechanisms that drive contact activation on non-physiological materials. Next, it will summarize and compare a number of bioassays that are available to investigate the contact system in health and disease. Finally, it will discuss findings from both fundamental and clinical studies on the contributions of contact system to disease.

O60 IMMUNOPHENOTYPING AND MOLECULAR DIAGNOSTICS IN MDS
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Accurate diagnosis in low risk myelodysplastic syndromes (MDS) necessitates additional sensitive and specific assays which can discriminate between MDS and non-MDS cytopenic patients, in particular in those cases without karyotypic anomalies, ringed sideroblasts or other major morphologic dysplastic features of granulocytes in peripheral blood films and dyserythropoiesis in bone marrow aspirates. The WHO classification 2008 contributes to a more refined classification and prognostication of myelodysplastic syndromes (MDS). Since flow cytometry (FC) can identify aberrancies in granulocytic, monocytic and erythroid lineages that are not recognised by cytology (WHO), FC might be instrumental in improving the diagnosis and classification of MDS. FC is even considered as co-criterion when regular diagnostic criteria are not met. Recently, within an international consortium (IMDSFlow), consensus was reached regarding cell sampling, handling and processing. Minimal combinations of antibodies that enable analysis of aberrant immunophenotypes and thus dysplasia are defined. Examples of aberrancies are altered numbers of myeloid progenitor cells (MPC), altered expression of myeloid/differentiation markers on MPC, maturing myeloid cells, monocytes or erythroid precursors as well as expression of lineage infidelity markers. Selected parameters concerning percentages and/or aberrancies of MPC, progenitor B cells, and side scatter properties of granulocytes might discriminate MDS from non-clonal bone marrow diseases. FC discriminates patients with >95% specificity and 70% sensitivity, with a flow cytometric score of at least 2 or higher, from non-MDS cytopenic patients. By adding aberrancies on progenitor and maturing erythroid cells an increase up >90% in sensitivity could be achieved. The number of flow cytometric aberrancies can be translated into a MDS flow-score. Within WHO and IPSS-R (revised) subgroups flow-scores are highly heterogeneous which might indicate separate disease entities. The MDS flow-score identifies patients with a worse clinical outcome after allogeneic stem cell transplantation. A significant increase in the MDS flow-score is observed between non-transfusion-dependent low/int-I risk patients and patients in progression to advanced MDS. Even in patients with uni-lineage dysplasia, e.g. RA-/-/RS, additional FC aberrancies are identified indicating multilineage dysplasia. Moreover, within the pure-RA-/-/RS subgroup only patients with infidelity marker expression on myeloid blasts are transfusion dependent. By multivariate analysis is was shown that the FCSS is an independent prognostic parameter after adjustment for validated prognostic scores such as IPSS, IPSS-R. Finally, flow cytometry is instrumental in predicting response to a standardized Epo/G-CSF regimen in lower risk MDS and to 5-azacitidine in higher risk patients with MDS. High-throughput DNA sequencing has significantly shown to contribute to diagnosis and prognostication in patients with MDS. Several independent groups show the biological and prognostic significance of genetic aberrations in MDS by identifying over 40 mutations in of > 100 genes tested. In 78-90% of cases with MDS at least one mutation was identified. In general, TET2, SF3B1, ASXL1, SRSF2, DNMT3A and
RUNX1 were mutated in >10% of cases. Many mutations were associated with higher risk MDS and associated with survival. In independent cohorts of patients, a top 12 of mutated genes in MDS could be identified. The status of 14 genes combined with conventional factors within the IPSS-R revealed a novel prognostic model separating patients into four risk groups. Moreover, a prognostic model based on only mutated genes might be at least as useful for the subclassification and prognostification in MDS. In conclusion, the standardisation of FC and molecular profiling in MDS is now available and contributes to improved diagnosis and prognostification. The integration of emerging new diagnostic tools might select patients to optimize treatment decisions in near future.

O61
UTILITY OF FLOW CYTOMETRY IN SYSTEMIC MASTOCYTOSIS
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Flow cytometric ex vivo analysis of human mast cells only became possible in the late 80’s. This was mainly due to the lack of mast-cell specific markers and the relatively low frequency at which these cells are typically present in bone marrow, peripheral blood and other primary tissues. In 1996 we established a well-standardized approach for the immunophenotypic identification of bone marrow mast cells based on combined staining for the CD117 and CD45 markers and acquisition of large numbers of cells (>5x10⁶). Since then, detailed characterization of the immunophenotypic features of human mast cells has been obtained by flow cytometry both in normal/reactive conditions and in patients with mastocytosis. Early studies already showed that bone marrow mast cells from almost every systemic mastocytosis patient display aberrant phenotypes. Among other markers, CD25, and to a lower extent also CD2, are identified as strongly associated with systemic mastocytosis. In fact the two markers have become in 2001 minor WHO diagnostic criteria for systemic mastocytosis. More recently, the value of CD25 has been strongly validated prospectively; conversely, CD2 did now appear to be as essential for diagnostic purposes as CD25, being absent in more than 25% of all systemic mastocytosis. This is also associated with overexpression of complement regulatory proteins (e.g. CD55 and CD59), activation (e.g. CD69) and degranulation markers (e.g. CD63 and CD203c). More interestingly, recent results have shown three clearly distinct immunophenotypic profiles of pathological bone marrow mast cells in systemic mastocytosis patients consistent with mature-activated, mature (non-activated) and immature mast cells. From the clinical point of view, these three immunophenotypic profiles were closely associated with specific subtypes of the disease; 1) the most common good-prognosis categories of systemic mastocytosis (indolent systemic mastocytosis with or without skin lesions including clonal mast cell activation syndromes), 2) well-differentiated systemic mastocytosis, and 3) systemic mastocytosis cases with poor-prognostic subtypes of the disease (aggressive systemic mastocytosis and mast cell leukemia), respectively. In detail, good-prognosis systemic mastocytosis cases typically showed a mature (e.g. FceRI+) immunophenotype associated with aberrant co-expression of both CD25 and CD2, and overexpression of mast cell activation markers such as CD69, CD64, CD63, CD203c and HLA-class II, in association in virtually every patient with the D816V KIT mutation. In turn, bone marrow mast cells from well-differentiated systemic mastocytosis cases typically lack CD2 and CD25, they show normal levels of expression of CD59, CD63, CD69 and CD203c; however, compared to normal mature mast cells, bone marrow mast cells from well-differentiated systemic mastocytosis cases typically showed abnormally high levels of expression of intracellular bcl2, carboxypeptidase and total tryptase. Bone marrow mast cells from mast cell leukemia and aggressive mastocytosis cases could be differentiated on immunophenotypic grounds from the previous subtypes of systemic mastocytosis because they showed bright expression of CD25, frequently in the absence of CD2, together with increased expression of markers of mast cell immaturity such as CD123 and HLA-DR, lower positivity for CD117, HLA-I, FceRI and intracellular enzymes (e.g. cytoplasmic tryptase and carboxypeptidase, an immunophenotypic profile typically associated with more immature mast cells. Of note, indolent systemic mastocytosis patients with multilineage involvement by the KIT mutation who typically represent one fourth of all indolent cases, but who are more prone to progress to more aggressive subtypes of the disease, also show more immature immunophenotypic features closer to those of the poor-prognosis systemic mastocytosis cases. In summary, these results confirm that bone marrow mast cells from systemic mastocytosis, despite being phenotypically different from normal bone marrow mast cells, they are still highly heterogeneous with clearly distinct phenotypic profiles. Such immunophenotypic features may therefore be used not only for the diagnosis and monitoring of systemic mastocytosis, but also for their subclassification into different subtypes associated with a distinct patient outcome.

O62
APLASTIC ANEMIA IN ASSOCIATION WITH A LYMPHOPROLIFERATIVE NEOPLASM
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Aplastic anemia (AA) may precede, co-occur or follow a lymphoproliferative neoplasm. The best molecularly clarified scenario is that of concurrent AA and unsuspected (occult) T-cell large granular lymphocyte leukemia. Several reported cases of AA and concurrent small B-cell lymphomas/leukemias and Hodgkin lymphomas suggest also a possible link to simultaneous or preceding AA that might be sought in an anti-neoplastic immunological attempt to “eradicate” the underlying malignant clone. The “immunoderegulatory” potential and the direct cytotoxicity of regimens used for lymphoma therapy might be able triggering AA in cases evolving after lymphoma treatment too. Alternative explanations of AA associated with lymphoproliferative disorders might be particular (immuno-)genetic patient backgrounds predisposing to both AA and lymphoid neoplasms or exposures to environmental factors, increasing the risk for both diseases. Finally, the most common causal relationship of AA and lymphoma is that of immunosuppression- or allogeneous hematopoietic stem cell transplantation-associated post-transplantational lymphoproliferative disorders in AA patients, who are treated in the respective manner. Since all above scenarios are differently (specifically) therapeutically approachable and accompanied by diverse outcomes, they should be actively sought for and diagnosed as precisely as possible. This presentation summarizes the current knowledge on associations between AA and lymphoproliferative neoplasms.
O64
RARE ANEMIAS DUE TO DISORDERS OF IRON METABOLISM
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Recent advances in understanding iron metabolism and systemic iron homeostasis have allowed identifying novel forms of rare anemias due to defects of proteins involved in iron absorption, utilization and recycling. These inherited disorders are usually characterized by microcytic and hypochromic anemia which reflects the low hemoglobin content per red cell secondary to a decreased intracellular iron availability. From a laboratory viewpoint these anemias may be classified as non-sideroblastic or sideroblastic. The former are heterogeneous and include:

a) defects of intestinal iron absorption due to excessive hepcidin production in iron refractory iron deficiency anemia (IRIDA). IRIDA is a recently recognized autosomal recessive entity, due to mutations of the serine protease TMPRSS6. The reported mutations reduce or abolish the TMPRSS6 ability to suppress hepcidin. Anemia is usually moderate but severely microcytic with low transferrin saturation and low/normal serum ferritin. The classic feature of IRIDA is refractoriness to oral iron and partial response to parenteral iron treatment.

b) recessive disorders of the transferrin receptor cycle. These include mutations of the iron carrier transferrin (atransferrinemia or hypotransferrinemia, mutations of the divergent metal transporter 1, DMT1 and of Six-Transmembrane Epithelial Antigens of Prostate 3, STEAP3). These defects impair the erythroblast iron uptake or utilization. The classic feature is moderate/severe microcytic anemia with high transferrin saturation and serum ferritin levels and evidence of systemic iron overload, especially in the liver.

c) defects of iron recycling as aceruloplasminemia due to recessive mutations of the ceruloplasmin gene. Since ceruloplasmin is an oxidase that cooperates with ferroportin to export iron from macrophages, hepatocytes and glial cells aceruloplasminemia presents as a syndrome characterized by systemic and liver iron overload, diabetes, retinopathy and mild/moderate normocytic/microcytic anemia associated with neurologic symptoms that appear in midlife. Iron accumulation occurs in the basal ganglia and for this reason the disease is classified as a type of neurodegeneration with brain iron accumulation (NIBIA).

Sideroblastic anemias are characterized by the presence of ringed sideroblasts at bone marrow smear examination after Perls’ staining. Sideroblasts accumulate iron in perinuclear mitochondria, hence showing a ring of iron localization. Acquired sideroblastic forms as Refractory Anemias with Ringed Sideroblasts (RARS), are a type of myelodysplasia, strongly linked to mutations of the spliceosome-associated factor SF3B1. Among the inherited sideroblastic anemias defects of mitochondrial iron utilization with reduced heme synthesis and rare defects of iron sulphur cluster biogenesis can be observed. The most common form is X-linked sideroblastic anemia, due to mutations of the first enzyme of the heme biosynthetic pathway, delta-aminolevulinic-acid synthase-2 (ALAS2). Some forms have mild/moderate anemia and improve after pyridoxine treatment. Among the pyridoxine-refractory forms there are recessive mutations of SLC25A38, a mitochondrial importer of glycine, a substrate of delta-aminolevulinic-acid, and extremely rare mutations of iron sulphur cluster biogenesis genes such as GLRX5 and ABCB7.

All rare atypical anemias related to iron metabolism should be differentiated from other more common causes of microcytic red cells that result from classic iron deficiency or thalassemia syndromes due to globin deficiency. Patient’s history and careful evaluation of hematologic tests and iron parameters allow distinction of these rare conditions from classic iron deficiency anemia or from iron restricted erythropoiesis. In most cases molecular studies are needed to allow the definitive diagnosis which is relevant for a correct treatment.

O65
HEREDITARY HEMOLYTIC ANEMIA DUE TO RED BLOOD CELL ENZYME AND MEMBRANE DEFICIENCIES
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There are a number of hereditary disorders which compromise the normal life-span of red blood cells (RBCs) of 120 days. The three main causes of hereditary hemolytic anemia comprise disorders of hemoglobin, the red cell membrane, or RBC metabolism. Patients with hereditary hemolytic anemia show signs of acute or chronic hemolysis. The degree of hemolysis and anemia is however highly variable. On one end of the spectrum there are patients who are severely anemic and even transfusion-dependent, whereas on the other end patients may display a fully compensated hemolysis without anemia. Additional clinical features frequently encountered are icterus, splenomegaly, and iron overload (even in absence of transfusions). Laboratory investigations show decreased hemoglobin levels, normal MCV, reticulocytosis, increased bilirubin and lactate dehydrogenase levels, and decreased levels of haptoglobin. The direct Coombs test is negative. In addition, RBC membrane disorders are characterized by changes in RBC morphology whereas enzyme disorders generally do not display an altered morphology. The one exception with regard to the latter is the prominent basophilic stippling that accompanies a deficiency of pyrimidine-5’-nucleotidase. For the differential diagnosis more sophisticated laboratory techniques are required (see below). RBC membrane disorders The RBC membrane consists of a cytoskeleton of spectrin that is linked to the plasma membrane by various proteins and protein complexes.1 The interaction between these proteins are of crucial importance for the integrity, flexibility, and deformability of the RBC and, hence, determines the cell’s ability to deliver oxygen to the tissues. Hereditary disorders affecting genes encoding RBC membrane proteins compromise these structural properties. They can roughly be divided in mutations of proteins that affect horizontal interactions in the cell membrane (i.e. the spectrin cytoskeleton) or mutations affecting vertical interactions (i.e. anchoring of the cytoskeleton to the membrane).2 In this way, and based on altered morphology, hereditary spherocytosis (HS) results from disturbed horizontal interactions, whereas hereditary elliptocytosis (HE) and hereditary pyropoikilocytosis (HPP) results from disturbed horizontal interactions. Hereditary RBC membrane disorders that present as ovalocytosis (i.e. South-east Asian Ovalocytosis or SAO) or stomatocytosis (HSt) comprise a group of abnormalities characterized by altered cellular ion and water content (annelopathies).3 RBC morphology remains an important tool in the differential diagnosis of RBC membrane disorders. The golden standard however is osmotic gradient ektacytometry. Basically, this technique measures RBC deformability under gradually changing osmotic conditions. The introduction of the Laser-assisted Optical Rotational Cell Analyzer (LoRRca, Mechatronics, Hoorn) has recently re-introduced this technique to the clinical laboratory. Another valuable addition to the diagnostic
The repertoire of RBC membrane disorders is the ability to confirm the diagnosis on the DNA level. Molecular analysis of membrane genes has long been hampered by the large and complex nature of the many different genes involved, and the fact that most mutations are private ones. With the advent of Next Generation Sequencing it is now possible to analyze the genes most commonly associated with RBC membrane disorders: SPTA1 (α-spectrin), SPTB (β-spectrin), ANK1 (ankyrin), SLC4A1 (Band 3), EPB41 (protein 4.1), EPB42 (protein 4.2), and RHAG (Rhesus-associated glycoprotein).

Establishing the molecular diagnosis will be particularly important in young children with congenital anaemia, transfusion-dependent patients, and in families with variable clinical expression or complex inheritance patterns. RBC membrane disorders Red blood cells require metabolic and reductive energy to fully perform their functional role. Metabolic pathways involved in cellular function and survival comprise anaerobic glycolysis, the hexose monophosphate shunt, glutathione metabolism and the nucleotide salvage pathway. These pathways serve to maintain (1) the integrity and flexibility of the red blood cell membrane, (2) hemoglobin in its reduced state, (3) the redox balance of the glutathione pool, and (4) maintenance of the adenine pool. Hereditary red blood cell enzymopathies are genetic disorders affecting genes encoding red blood cell enzymes. They cause a specific type of anemia designated hereditary nonspherocytic hemolytic anemia (HNSHA). Enzymopathies are commonly associated with normochromic normocytic hemolytic anemia. In contrast to other hereditary red cell disorders such as membrane disorders or hemoglobinopathies, morphological abnormalities of the red blood cell are absent. Notably, some enzyme deficiencies are associated with systemic (non-hematological) manifestations such as neurological dysfunction, mental retardation, myopathy and susceptibility to infection.14 The laboratory diagnosis of an RBC enzymopathy involves the determination of specific enzyme activity. Decreased activity is indicative of an enzyme deficiency. Importantly, there is no correlation between residual enzymatic activity and the patient’s clinical picture. Preferably, a suspected RBC enzyme disorder is confirmed by identifying the causative mutation on the DNA level. The most common enzyme disorders are deficiencies of glucose-6-phosphate dehydrogenase and pyruvate kinase. However, there are a number of other enzyme disorders, often much less known, causing HNSHA. These disorders are rare and often underdiagnosed.

**O66 RED BLOOD CELL DISORDERS**

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External Quality Assessment (EQA) is an intrinsic part of Best Practice. Besides international assessment schemes also local external assessment schemes are available for a variety of laboratory techniques used for the diagnosis of hemoglobin variants, red blood cell enzyme activity measurements like G6PD, hemocytometric analysis for hemoglobinopathies and molecular diagnostics of thalassemia mutations and mutations causing Hb variants. Participation in External Quality Assessment is encouraged and essential for laboratories already accredited or seeking accreditation to international standards, such as for example ISO 15189 or equivalent (1,2). International assessment schemes are available from UKNEQAS as far as G6PD and DNA analysis for hemoglobinopathies are concerned, the latter is already available for more than 10 years now and attended by many laboratories throughout Europe involved in molecular diagnostics for hemoglobinopathies. Also local assessment schemes like for example a hemoglobin variant scheme organized by the SKML (Stichting Kwaliteit Medische Laboratoria) in The Netherlands, are attended by over 50 local hematological laboratories. Issues as which assessment schemes to apply to, what kind of problems are met and which improvements are made will be discussed.

**O67 COAGULATION**

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**Introduction:** High quality in the medical laboratory is prerequisite for the delivery of reliable test results to the physician. This covers not only the analytical phase of the testing process, but also the pre- and post-analytical phase. During the presentation a common observation in the haemostasis laboratory will be discussed: an unexpected prolonged activated partial thromboplastin time (APTT). Important quality aspects of the total testing process will be discussed and examples from the ECAT external quality assessment programme will be discussed. It will become clear that quality does not only mean accurate test results but also proper action in both the pre- and post-analytical phase. Lastly, an comprehensive approach for diagnostic external quality assessment will be discussed.

**O68 BLOOD CELL MORPHOLOGY**

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Conventional proficiency testing (or external quality assessment) focuses on the analytical phase of laboratory testing. An established model of external quality assessment (EQA), as offered by the UK National Quality Assessment Scheme for General Haematology (UK NEQAS (H)), performance assesses the laboratory as a whole, with no assessment of the performance of the individual practitioner. This provides a challenge for the provision of blood cell morphology EQA, where the skills of the individual practitioner are key but the overall interpretation of the case may involve a range of staff and the results of other diagnostic investigations. Modern automated cell counters have rendered the need for routine examination of large numbers of blood smears unnecessary, removing the training ground of reviewing numerous normal slides; however, the blood film remains an essential part of diagnosis (1) and forms part of the quality control of the output of automated analysers, as features may be observed on the film that correlate with conditions that affect the validity of the automated blood count, e.g. the presence of cold agglutinins, platelet satellitism, platelet clumps and fibrin strands.

Pre-analytical errors common to all diagnostic testing (2), including wrong patient, inadequate or misleading clinical information, incorrect specimen type, incorrect labelling and data entry errors, are equally applicable to blood film morphology. In addition, the quality of the film preparation affects the distribution of the cells in a manually prepared wedge film and the slide preparation step itself allows an additional opportunity for misidentification of the specimen. Film preparation is time critical and changes in cell morphology become apparent within a few hours of storage in EDTA. A film made directly from non-anticoagulated blood will show different features from one made from EDTA blood and conditions associated with a high haematocrit or severe lipaemia make film preparation difficult. Once the blood smear is made, artefacts may be introduced in the process of fixation and staining, affecting cell appearances (3).
Blood film morphology is an area of laboratory medicine where the post analytical phase is integral to the process, from the ‘what would you do next?’ scenario to the provision of a suggested diagnosis, depending upon the extent of information available. The reporting of blood film morphology requires considerable haematological knowledge and, where undertaken by laboratory scientists, should be governed by rules based protocols on the action points that require referral to senior scientific or clinical staff and what requires immediate communication to the requesting clinician.

So what can EQA in cell morphology assess and where might the gaps lie? In the ‘analytical’ phase, much depends upon the objectives of the EQA scheme design, in particular the minimum data set provided with the blood smear. If the programme intends to evaluate morphology skills, then only the briefest of case details need be provided in the first instance. Full case history with additional pathology results must be supplied in the final report, for the maximum educational benefit. The alternative is to provide full clinical details with the smear at distribution. This is more true to laboratory practice but prior knowledge of full details has been shown to influence the choice of morphological features reported.

With the provision of full clinical details, the exercise becomes an interpretative case study, equally valid from an educational point of view but of different impact. The use of scoring to evaluate performance in blood film morphology is an area for much debate. It is recommended (4) that the evaluation report should include a breakdown of the responses given by the participants and an expert comment but scoring is only regarded as essential for the differential count, if requested. Opinion from participants has shown the desirability of rapid feedback on the nature of the case and this may conflict with the aspiration of a high quality, reflective narrative.

UK NEQAS (H) provides morphology skills EQA in both glass slide and digital format in its morphology suite of programmes. The glass slide exercises are sent to the laboratory as a whole and anecdotal evidence shows that participant laboratories approach the exercises in different ways, with individual or collective review by scientific and/or clinical staff, both before result submission and once the report is received. Since the cases cover a wide range of clinical conditions, from the commonplace to the very unusual, the exercises both maintain and extend skills. Digital morphology exercises for continuing professional development are provided to individually registered practitioners through a secure web portal. Participants view high quality, virtual slides, each prepared from stitched images, reporting their observations and responding to a brief questionnaire on their next actions and the probable diagnosis.

One pre analytical area that is rarely examined in EQA is that of the staining of the blood smear. The introduction of automated blood smear preparation and staining should improve standardisation but may result in a loss of skills and knowledge. UK NEQAS (H) occasionally distributes an unstained blood film for the participant to stain and report. An observation in these cases is that the proportion of slides reported as of unsatisfactory stain quality is greater than when a stained film is distributed. The major difficulty in assessing stain quality is the establishment of a target or reference against which to compare performance.

The future challenges to blood film morphology EQA include the accommodation of cell recognition systems, of which CellaVision® is an example. This system automatically classifies cells from a blood smear, displaying the results for validation by the operator. The operator may review not only unclassified cells but also override the classifications made by the system (5). This is a new way of working, which brings the opportunity for standardisation but also variability according to the skill and opinion of the operator.
POSTER SESSION I

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HAEMATOLOGICAL PROFILE OF HOSPITALIZED PATIENTS INFECTED BY WEST NILE VIRUS

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Introduction: West Nile Virus (WNV) is an RNA virus that belongs to the Flaviviridae family. Humans are mainly infected through mosquito (aedes spp) bites. The aim of the study was to evaluate the haematological profile of hospitalized patients infected by WNV and to investigate if there is any parameter with positive predictive value in order to help the diagnosis in the Emergency Room.

Methods: We performed a retrospective review (period August – September of the years 2012 and 2013) of the haematological data recorded on admission of 52 hospitalized patients (31 men, 21 women; with a median age of 68 years) infected by WNV (referred as Positive group), as well as of the corresponding data of 27 hospitalized patients (19 men, 8 women; with a median age of 74 years) with suspected infection with WNV, but finally diagnosed as negative (referred as Negative group). The diagnosis in both groups was confirmed by the reference laboratory. Full blood count was performed with Sysmex XT-2000i analyzer. Statistical analysis: Descriptive statistics refer to means and their SD values as well as to median. Group comparisons were performed by the Mann-Whitney U test. The diagnostic predictive efficiency was studied by constructing receiver operating characteristic (ROC) curves and calculating the area under the ROC curve (AUCROC).

Results: The two groups (Positive vs Negative) did not present a statistical significant difference in white blood cell count (9.73±4.14 vs 10.52±4.77; 9.20 vs 9.94 x10³/μL; p=0.627), in neutrophils (72.9±10.9 vs 77.1±12.9; 74.4 vs 79.1% p=0.091) and in platelets (178±58 vs 203±99; 175 vs 206 x10³/μL; p=0.226). The two groups differed statistically significantly in lymphocytes (19.2±9.6 vs 15.1±10.3; 17.2 vs 12.8%; p=0.042), in monocytes (7.4±2.6 vs 5.8±2.5; 7.4 vs 5.7%; p=0.021) and in the population of activated lymphocytes, classified by the analyzer as “other” (1.02±1.01 vs 0.09±0.2%; 0.6 vs 0.0%, p=0.001). The ROC curves indicated that among the study parameters, “other” presented a very good diagnostic efficiency (AUCROC =0.897; p=0.0001) in distinguishing Positive group with sensitivity = 0.826 and 1-specificity = 0.148 at the cut-off point ≥30%. The corresponding positive and negative predictive values were 0.91 and 0.71, respectively.

Conclusions: The parameter “other” indicating activated lymphocytes, provided by Sysmex XT-2000i analyzer, has a very good predictive value for WNV infection and thus can help the diagnosis in the Emergency Room.

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DIAGNOSTIC PERFORMANCE OF THE VARIANT LYMPHOCYTE BY BECKMAN COULTER LH780 HEMATOLOGY ANALYZER IN HEMORRHAGIC FEVER PATIENTS

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Introduction: Blood cells in Dengue Hemorrhagic Fever (DHF) patients can be changed in function, morphology and numbers, especially an alteration of lymphocyte to be variant lymphocyte. Methods: Retrospective study in 50 patients admitted in Taksin Hospital, Medical Service Department, Bangkok, who were definite diagnosed and recorded by clinician as DHF patients was performed. Variant lymphocytes flagging reported by hematology analyzer Beckman Coulter LH 780 with VCS technology™, were compared with those of variant lymphocytes detected by microscopic white blood cell differential results and CellaVision DM96. In order to reduce inter-observer bias from blood smear differential count for variant lymphocyte reporting, those results of CellaVision DM96 which expertise-edited were used as a standard method. Results: To evaluate the diagnostic performance of variant lymphocyte flagging by Beckman Coulter LH 780 in DHF patients and classify types of variant lymphocytes observed in these patients, numbers and types of variant lymphocyte were analyzed. The findings of sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of variant lymphocyte detection by using variant lymphocyte flagging alone were 21.1%, 100%, 100% and 49.6%, respectively. Whereas 96.0% of sensitivity, 98.4% of specificity, 98.0% of PPV and 96.9% of NPV were observed in using variant lymphocyte combined with other flagging (monocytosis%, mononcytosis #, monoblast, lymphoblast and lymphocytosis). Furthermore, 80.5% plasmacytoid lymphocyte, 18.3% mononcytoid lymphocyte and 1.2% blastoid lymphocyte were observed in these DHF patients. Conclusions: The present study shows that to increase correct detection of variant lymphocytes, not only variant lymphocyte flag but also mononcytosis %, mononcytosis #,monoblast, lymphoblast and lymphocytoisis flags should be considered.

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BLOOD CIRCULATING ATYPICAL LYMPHOCYTES DETECTION AND ENUMERATION USING THE CYTODIFF®MONOCLONAL ANTIBODIES PANEL ON HEMATOFLOW®PLATFORM

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Introduction: Atypical lymphocytes (AL; i.e viruscytes, reactive lymphocytes) are reactive T cells that appear in the blood during viral conditions such as infectious mononucleosis, CMV infection, HIV, viral hepatitis, toxoplasmosis and sometimes are drug induced. The count of atypical lymphocytes is traditionally performed using manual or digitalized microscopy in response to hematology analyzers flags or lymphocytosis. For 3 years we work with the HematoFlow platform in our routine practice using the Cytodiff® reagent to control the DxH800 “Variant lymphocytes” and “blasts” flags. Cytodiff was not designed to detect and recognize atypical lymphocytes which are not part of the 9 population differential IVD-validated. A cytometric approach demonstrated that reactive T cells appear larger and express more CD45 and CD2 that resting lymphocytes. We compared the results obtained by Cytodiff in a CD2/CRT/2/CD45 plot with microscopic results using Passing Bablock and Bland Altman analysis. Methods: Blood films of patients with “Variant lymphocytes” or “blasts” DxH800 flags were reviewed. Cases with more than 3% of AL counted by microscopy were selected. A CD45/CD2 plot based on T and Natural Killer lymphocyte population was added in the standard Cytodiff analysis protocol proposed by Beckman-Coulter. AL enumeration was based on their high CD45/CD2 expression. Coefficient of variation (CV) of cytometric enumeration was evaluated on 5 independent
analysis of the same sample. Microscopy CV was evaluated by five 100 cells manual differentials realised by two technologists. Viral infection was confirmed by serology. Results: 57 specimens were selected with following infectious diseases: 22 EBV; 14 CMV; 1 HHV; 1 dengue, 5 HHV6, 3 viral hepatitis, 1 toxoplasmosis and 1 BK-JC virus. Respective CV of the microscopic and cytometric analysis of AL were 49% and 10%. Passing Bablok curve was y = -0.0994798 + 0.929865 * x. Bland Altman analysis shows an interval of 0.2 ± 1.3%. Conclusions: Cytometry has a five times lower CV and is at least as good as microscopy, it can be used as a surrogate method for AL count in the routine laboratory. Cytometry provides quick information that can orientate diagnostic without need of microscopy trained technicians. To fulfill quality standard agreement, internal and external quality controls for AL count by cytometry are required.

106 SLIDE REVIEW CRITERIA ON THE SYSMEX XN1000 IN A PRIVATE LABORATORY IN MUMBAI INDIA
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Introduction: Bhide Laboratory services has been using automation in hematology since 1974. With centralized laboratory and 7 collection centers, laboratory caters to walkin patients. Patient population is a mix of routine health checkup plans and with some disease. Dengue and malaria are common findings. Other red cell pathologies like Iron deficiency anaemia is very common. XN1000 offers several new features not available in the previous models. It transmits more than 200 parameters to the host for every sample. Methods: 556 consecutive EDTA whole blood samples were analyzed on XN1000. A host PC with a middleware was used to capture data. A set of rules for slide review were built in middleware to analyze data. The XN1000 has quantifiable Q flags for sample abnormalities like Blasts cells, Atypical lymphocytes, RBC and Platelet clumps. Threshold of these Q flags is user definable from 100 to 300 units. In this study the threshold was set to 200. Over a period of 3 months proceeding this study several observations were made and a set of rules was finalized. These set of rules was included in middleware which was applied to data captured. Along with printout of the instrument, middleware also printed a report with flags, indicating which slides will require review and what is to be expected from microscopy. Slides of 556 samples were reviewed manually over 4 working days and compared with the findings of the middleware and predefined rules. Results were analyzed to find sensitivity and specificity of rules defined. Conclusions: Specificity of the system was 81.24%. The effective slide review rate was 18.76%. Sensitivity of the system was 99.89%. The system picked up almost all samples with some abnormality on smear and at the same time samples which required slide review were not missed.

108 BLASTS COUNT WITH HEMATOFL ow PLATFORM: 3 YEARS' EXPERIENCE IN A MIDDLE-SIZED ACADEMIC HOSPITAL.
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Introduction: Leucocyte differential count is a central analysis in hematology laboratories. Analysers allow identifying five normal blood cells populations (neutrophils, basophils, eosinophils, monocytes and lymphocytes). Microscopy is traditionally used to characterize other cell populations by morphology. HematoFlow, implemented in our laboratory, is a new concept that combines haematology analysers with a cytometry platform, allowing the detection of nine blood cells populations: usual leucocyte differential count including blasts, immature granulocytes, B lymphocytes and NK/T lymphocytes. We reviewed blast cell counts generated by hematoFlow between September 2010 and August 2013 to determine accuracy, interferences and clinical impact. Methods: HematoFlow concept includes DxH800 blood cell analyser, FP1000 sample preparatory, FC500 cytometer and CytoDiff reagent, 5 colours 6 monoclonal antibodies combination (CD45, CD19, CD16, CD36, CD2, CD294) (Beckman Coulter). HematoFlow process takes into account 20000 cells while usually 100 to 200 cells are observed by microscopy. To calculate blast count accuracy, 17 clinical samples (blast range : 0.3-2.5%), including 7 cytopenic (< 4x10^9/WBC/L) were analysed 7 times by cytometry while blood films were counted 4 times for 100 cells by 3 technologists. Results: In 3 years, we performed 403.103 leucocyte differential counts (daily range 35-61, mean 44.2) that generated a CytoDiff in 8.5% of the cases for a total of 34.461 CytoDiff, 9 blood cell populations differential counts. Internal coefficient of variation (CV) for blast counts was 20% for normal samples and 34% for cytopenic samples. By microscopy, we found a CV of 299% for normal samples. Interference occurs when other cells move inside the blast gate: CD36 negative monocytes, dysplastic neutrophils (hypogranularity and loss of CD16 expression) and B lymphocytes with a weak expression of CD19 (for example in B-CLL) can be found in the blast gate. These complicated cases are submitted to the biologist. We detected and followed accurately blasts in 27 primary and 22 secondary acute leukemia, 34 myeloproliferative/ myelodysplastic diseases with blast excess, 74 post-chemotherapy bone marrow regeneration and in 52 sepsis. Conclusions: HematoFlow provide better sensitivity for blasts cell count than microscopy, due to the high number of cells counted and the phenotypic informations provided. In leucopenic samples a “buffy coat” is no longer necessary.

110 THE HEMATOLOGY LABORATORY IN BLOOD DOPING (BD): 2014 UPDATE ON THE ATHLETE BIOLOGICAL PASSPORT (ABP)
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Introduction: Blood doping (BD) is the use of Erythropoietic Stimulating Agents (ESAs) and/or transfusion to increase aerobic performance in athletes. Direct toxicologic techniques are insufficient to unmask sophisticated doping protocols. The Hematological module of the ABP (World Anti-Doping Agency), associates decision support technology and expert assessment to indirectly detect BD hematological effects. Methods: The ABP module is based on blood parameters, under strict pre-analytical and analytical rules for collection, storage and transport at 2-12°C, internal and external QC. Accuracy, reproducibility and interlaboratory harmonization fulfill forensic standard. Blood samples are collected in competition and out-of-competition. Primary parameters for longitudinal monitoring are: - hemoglobin (HGB); - reticulocyte percentage (RET); - OFF score, indicator of suppressed erythropoiesis, calculated as [HGB(g/L) * 60 -RET%]. Statistical calculation predicts individual expected
limits by probabilistic inference. Secondary parameters are RBC, HCT, MCHC-MCH-MCV-RDW-IFR. ABP profiles flagged as atypical are review by experts in hematology, pharmacology, sports medicine or physiology, and classified as: - normal - suspect (to target) - likely due to BD - likely due to pathology.

**Results:** Thousands of athletes worldwide are currently monitored. Since 2010, at least 35 athletes have been sanctioned and others are prosecuted on the sole basis of abnormal ABP, with a 240% increase of positivity to direct tests for ESA, thanks to improved targeting of suspicious athletes (WADA data). Specific doping scenarios have been identified by the Experts (Table and Figure).

<table>
<thead>
<tr>
<th>Change in HGB</th>
<th>Change in RET</th>
<th>Doping scenario</th>
<th>Possible pathology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Increased</td>
<td>Increased</td>
<td>Recent or ongoing ESA stimulation</td>
<td>None</td>
</tr>
<tr>
<td>Increased</td>
<td>Decreased (high OFF)</td>
<td>Suppressed erythropoiesis after ESA cessation</td>
<td>Primary or secondary erythrocytosis</td>
</tr>
<tr>
<td>Decreased</td>
<td>Increased or unchanged (low OFF)</td>
<td>Blood withdrawal</td>
<td>Blood loss, in vivo hemolysis</td>
</tr>
<tr>
<td>Increased during a multi-day race</td>
<td>Decreased or normal</td>
<td>RBC reinfusion</td>
<td>Severe hemocencentration</td>
</tr>
</tbody>
</table>

**Figure.** Typical HGB and RET profiles in two highly suspicious athletes. A. Sample 2: simultaneous increases in HGB and RET (likely ESA stimulation) in a male. B. Samples 3, 6 and 7: “OFF” picture, with high HGB and low RET in a female. Sample 10: normal HGB and increased RET (ESA or blood withdrawal).

**Conclusions:** ABP is a powerful tool for indirect doping detection, based on the recognition of specific, unphysiological changes triggered by blood doping. The effect of factors of heterogeneity, such as sex and altitude, must also be considered.


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**UF-1000i: VALIDATION OF THE BODY FLUID MODE FOR COUNTING CELLS IN BODY FLUIDS**

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**Introduction:** The UF-1000i is the first fully automated urinalysis analyzer developed by Sysmex containing a urine mode and a body fluid (BF) mode. We evaluated the new body fluid mode on the UF-1000i urinalysis analyzer for counting blood cells in CAPD, ascites and pleural fluids. **Methods:** We collected 154 (60 ascites, 33 pleural, 61 CAPD) body fluid samples and compared the results of the UF-1000i BF mode with the Fuchs-Rosenthal channel and the XN-1000 BF mode. Linearity, carry over and precision were also assessed. **Results:** Method comparison results showed very much acceptable WBC agreement between UF-1000i and counting chamber (y=1.27x + 3.13, n=135, r=0.99) and between UF-1000i and XN-1000 (y=1.15x + 0.31, n=135, r=1.00). Comparison between the UF-1000i and both comparison methods showed excellent agreement for RBC counts. Overall results were better when UF-1000i was compared with the XN-1000 than with the Fuchs-Rosenthal chamber. The Lower Limit of Quantitation was defined at 9x10⁶ WBC/L and at 25x10⁶ RBC/L. Linearity was excellent for both WBC (r=1.00) and RBC (r=0.99). Carry over was negligible and never exceeded 0.01%. In one sample, a high discrepancy was observed between WBC results for both automated analyzers and the counting chamber due to interfering factors such as bacteria and yeast cells. This led to a false increased WBC count on both automated systems. **Conclusions:** The UF-1000i BF mode offers rapid and reliable total WBC and RBC counts for initial screening of CAPD, ascites and pleural fluid, and it can improve the workflow in a routine laboratory; however, when using automated analyzers, the inspection of scattergrams is required to ensure the most accurate results.

**114 MENINGIOMA 1 (MN1) EXPRESSION: REFINED RISK STRATIFICATION IN ACUTE MYELOID LEUKEMIA WITH NORMAL CYTOGENETICS**

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**Introduction:** Prognostic stratification of cytogenetically normal acute myeloid leukemia (CN-AML) is a point of intensive research. We aim to determine the prognostic importance of the meningioma 1 (MN1) gene expression levels in CN-AML. **Methods:** One hundred patients with AML were diagnosed, MN1 expression were analyzed using quantitative real time (QRT) PCR. **Results:** High expression was detected in 48 (48%) patients (range: 2.35-31.99, mean: 13.9 ± 8.49) in comparison to 52 (52%) patients with low expression (range: 0.02-2.3, mean: 0.68 ± 0.77). The course of the disease in patients with high MN1 expression was unfavorable. Patients with high MN1 expression was associated with significant low complete remission (CR) rate (62.5% vs. 88.4%, high vs. low MN1, P=0.001) and high mortality rate (75% vs 46.1, P=0.03). AML patients with high MN1 expression tended to be refractory (37.5% vs 19.2, P=0.00) and relapse risk (54.1% vs 23%, P=0.02). Multivariable analysis confirmed high MN1 expression as an independent risk factor for disease free survival (DFS) and overall survival (OS). **Conclusions:** MN1 over expression independently...
**116 COMPARISON OF AUTOMATED BLOOD CELL COUNTS BETWEEN ADVIA 120 AND SYSMEX XT 4000 i IN BREAST CANCER PATIENTS AND HEALTHY CONTROLS**

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**Introduction:** Breast cancer patients have become areas of great interest in Thai. Breast cancer is usually treated with surgery, which may be followed by chemotherapy or radiation therapy, or both. Correlations between survival outcomes and complete blood counts are of interest. In the clinical laboratory, Advia 120 and Sysmex XT 4000 i are in routine used and quantification remain challenging in breast cancer. This study aims to determine the correlation between 2 hematology analyzers; Advia 120 and Sysmex XT 4000 i and to compare parameters, which are different between the breast cancer patients and healthy normal controls.

**Methods:** A total of 100 EDTA blood samples from breast cancer at Mahavajiralongkorn Cancer Center and 100 blood samples from healthy controls were analyzed in the manual mode with Sysmex XT 4000 i and Advia 120. The Student’s t-test and Pearson correlation coefficient were tested by using SPSS 17.0.

**Results:** Two analyzers exhibited very good correlation for fifteen parameters, WBC, RBC, HGB, Hct, MCV, MCH, PLT, MPV, absolute neutrophil count, neutrophils, absolute lymphocyte count, lymphocytes, absolute monocyte count, monocytes, and eosinophils in patients with breast cancer. The correlation coefficients (r) of these parameters were 0.993, 0.983, 0.808, 0.798, 0.984, 0.988, 0.977, 0.785, 0.993, 0.983, 0.902, 0.991, 0.777, 0.966 and 0.911, respectively. Consideration on blood cell parameters between healthy controls and breast cancer patients, by Advia 120, the significant differences were shown in all parameters (p<0.05) except PLT, absolute neutrophil count, absolute monocyte count, absolute eosinophil count, eosinophils, and large unstained cell (LUC). By using Sysmex XT 4000 i, all observed parameters were statistically different (p<0.05) except MCHC, cellular hemoglobin concentration mean (CHCM), hemoglobin distribution width (HDW), neutrophils, absolute monocyte count, absolute basophil count. **Conclusions:** The fifteen parameters were acceptable, no significant bias, and good correlation. The accuracy and precision of the most parameters for the Sysmex XT 4000 i and Advia 120 were satisfactory even test in patients with breast cancer.

**118 RDW-CV, IG COUNT AND MO-X OBTAINED FROM NEW AUTOMATIC HEMATOLOGY ANALYZER SYSMEX XN-2000 PROVIDE USEFUL INFORMATION FOR DIAGNOSIS AND PROGNOSIS OF DIC**

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_Asan Medical Center Seoul, South Korea_

**Introduction:** Disseminated intravascular coagulation (DIC) is characterized by systemic activation of blood coagulation and inflammation which results in multiple organ dysfunction syndrome. In DIC, monocytes and neutrophils are activated, and RBCs are damaged. Newly developed automatic hematologic analyzer XN-2000 (Sysmex, Kobe, Japan) could detect the changes of blood cells in DIC. We investigated the 36 routine CBC items and 57 cell population data (CPD) provided by XN-2000 to discover items reflecting the changes of blood cells in DIC patients. **Methods:** A total 118 patients including 60 DIC patients, 58 non-DIC patients and 280 healthy controls were included. The items were compared between DIC patient group and healthy controls with receiver operating characteristic (ROC) analysis, and area under curve (AUC) over 0.90 were primarily selected. Among selected items, the items with AUC over 0.75 in comparison between DIC patient group and non-DIC patient group were finally identified. **Results:** RDW-CV (coefficient variance of red cell distribution width) >15.7%, IG count (immature granulocyte) >0.5/µL and Mo-X (lateral scattered light intensity of monocyte area on WDF scattergram) >120.7 were discovered for the diagnosis of DIC, and the AUC (95% CI) from ROC analysis between DIC patient group and non-DIC group were 0.909 (0.843-0.954), 0.925 (0.862-0.965) and 0.788 (0.703-0.858), respectively. The sensitivities and specificities of the selected three items for diagnosis of DIC were 60.8% and 88.0% for IG counts, 80.8% and 75.9% for Mo-X and 86.7% and 79.0% for RDW-CV, respectively. The combination of RDW-CV and IG count showed the best sensitivity (95.0%) and specificity (75.9%). In multivariate analysis for 28-day mortality, RDW-CV >16% was a significant predictor (p=0.047), and also revealed poor survival in Kaplan-Meier survival analysis (p=0.010). **Conclusions:** The RDW-CV, IG count and Mo-X showed better diagnostic power for diagnosis of DIC than any other CBC items including platelet count, and are expected to give valuable information for diagnosis and monitoring of DIC without additional costs and time consuming. RDW-CV could be a prognostic marker for overt DIC patients.

**120 EVALUATION OF SYSMEX XN-2000 AND XE-2100 IN UMBILICAL CORD BLOOD**

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**Introduction:** Cord blood (CB) reflects the hematologic status of neonates and is important for hematopoietic stem cell transplantation. We compared Sysmex XN-2000 and XE-2100 (Sysmex, Kobe, Japan) in CB specimens. **Methods:** We compared CBC parameters and white blood cells (WBC) differentials between the two analyzers in 160 CB specimens. Flagging performances of blasts, abnormal/typical lymphocytes, immature granulocytes...
and/or left-shift (IG), and nucleated RBC (NRBC) counts were compared with slide reviews between the two analyzers. **Results:**

The two analyzers showed acceptable correlations for most of the CBC parameters and WBC differentials. Compared with XE-2100, XN-2000 showed better efficiency for flagging performances for blasts, abnormal/ atypical lymphocytes, and IG. XN-2000 showed better efficiency for flagging blasts than XE-2100 (81.4% vs. 41.3%, P < 0.0001). XN-2000 showed remarkably increased specificity (99.2%) of blast flag, compromising its sensitivity of blast flag. For abnormal/atypical lymphocytes, XN-2000 and XE-2100 showed excellent sensitivity (92.3% vs. 87.9%) although both analyzers showed compromising sensitivity (11.4% vs. 2.5%, P < 0.0001). XN-2000 and XE-2100 showed useful specificity for IG flag, compromising their specificity (18.8% vs. 44.9%, P < 0.0001). Both analyzers showed interchangeable performances for NRBC flag and showed good correlations with manual NRBC counts. **Conclusions:**

This study highlighted the differences of flagging performances between Sysmex XN-2000 and XE-2100 in CB specimens. These interesting findings would require further confirmation in the other studies using various clinical specimens.

**122** MEAN PLATELET VOLUME AND MEAN NEUTROPHILIC VOLUME AS PREDICTORS OF THE PRESENCE AND SEVERITY OF DIABETES MELLITUS

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All India Institute Of Medical Sciences New Delhi, India

**Introduction:** Introduction: Diabetes Mellitus (DM) has been associated with increased risk of both micro and macrovascular complications in which platelet plays a pivotal role. Mean platelet volume (MPV) is an indicator of its function. MPV and Mean Neutrophilic volume (MNV), another useful parameter can be easily determined on routine automated hemograms. The objective of this study is to compare the MPV and MNV in patients with DM, Pre-diabetes or Impaired DM (IDM) and non-diabetic controls.

**Methods:** This is a retrospective observational study done in the Department of Laboratory Medicine, All India Institute of Medical Sciences, New Delhi between September-2012 to September-2013. A total of 40 healthy controls and 153 patients were selected and sub-grouped as Impaired Diabetes Mellitus (IDM) (Gr A), DM (Gr B) and DM with complications(Gr C). Hemogram from both cases and controls were run on Beckman Coulter LH-750 and Volume Conductivity Scatter(VCS) parameters were noted. Fasting and post-prandial glucose were estimated in blood chemistry analyser. **Results:** The age and gender ratio of the cases were comparable to control.(p>0.05). MPV was significantly different between cases and control (10.6 ± 1.8 vs 7.01±1.4, p<0.05) as well as between groups Gr A vs B (p<0.05), A vs C (p<0.05) and B vs C (p<0.05). Similarly, MNV was statistically significant between cases and controls (146.7±14.6 vs 140.57±7.7, p< 0.05) and also among the DM groups Gr A vs C (p< 0.05) and B vs C (p<0.05). Cut-off value for the prediction of prediabetes, diabetes and diabetes with complication was calculated and elucidated in table no:1 **Conclusions:** MPV was significantly raised in the cases when compared to control group and lower in the IDM when compared to DM as well as with DM with complications. MNV was significantly raised in the overall DM group as well as in the DM with complications when compared to IDM and DM. Our study suggests a potential role of in subjects with diabetes. MNV and MPV is strongly and independently associated and can predict the presence and severity of diabetes.

**124** NO NEED FOR MICROSCOPY OR FLOW CYTOMETRY TO QUANTIFY MYELEMA IN DAILY HAEMATOLOGY LABORATORY PRACTICE.

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**Introduction:** The presence of immature granulocytes (IG) in the peripheral blood reflects active bone marrow response to different states, including sepsis, post-chemotherapy response or myeloproliferative disorders. Conventionally, this myeloma is quantified on stained blood films by microscopy in promylocytes, myelocytes, metamyelocytes and band cells. New hematology analysers are able to detect and quantify myeloid immaturity. The DxH800 (Beckman Coulter) classifies the immature cells as EGC (Early Granulocytes Cells). These analysers are linked to allow cytometer, which allows defining the percentage of IG (CD16dim) with SS/CD16 cytometry plot. The objective of this study is to compare those IG count methods in terms of efficiency, accuracy and costs for the laboratory. **Methods:** IG counts, selected by “IG” alarm during 3 months, were compared on 117 samples by microscopy, both DxH800 automates and by flow cytometry (FC500) with Passing Bablok (PB) and Bland Altman (BA) analysis. The coefficient of variation was determined on 20 samples, between both DxH800 and between 3 different technologists by microscopy (100 cells counts, 5 times). **Results:** Regarding microscopy, 81/117 samples had IG > 3% of leucocytes. Technologists’ coefficients of variation (CV) were respectively 34.5, 37.6 and 37.4%. The inter-technologist CV was 43.78%. Mean CV of both DxH800 was 10%. There is a moderate difference between the two DxH800 (PB: y = -0.0920539 + 0.973064 x ; BA: 0.1 ± 2.3). EGC results sufficiently correlated with microscopy (PB: y = -0.06835821 + 0.910448 x, BA: -0.04 ± 0.46). A good correspondence was found between EGC count by DxH800 and IG by FC500 (p=0.01). **Conclusions:** This study shows that EGC counts delivered by DxH800 analysers can be considered as a good surrogate of microscopic blood smear IG enumeration and can be used in everyday practice. Even in the absence of internal and external control, EGC count is at least as efficient as microscopy and flow cytometry. Microscopy is a time consuming technique which requires well trained technologists. Flow cytometry is an expensive monoclonal antibodies-based method. Reporting EGC values from haematology analysers represents no supplementary cost for the laboratory. However, the blood smear is systematically reviewed for myeloid dysplasia exclusion.
Reliable automated blood cell characterization and quantification remain challenging in pathologic samples, whereas slide reviews due to unnecessary flagging should be avoided. The Sysmex XN series provides the possibility to automatically count the immature granulocyte fraction (%IG) in the peripheral blood. The %IG includes promyelocytes, myelocytes and metamyelocytes (blasts and band cells are not included). After applying a specific lysis agent (Lysercell WDF), the %IG is measured in the WDF channel and differentiation is made based on granularity (side scatter) and nucleic acid content (side fluorescence by the Fluorocell WDF reagent). In the present study the %IG counted by the XN series was compared with automated microscopic differentiation of 500 white blood cells (WBC)

Methods: Peripheral blood samples from 103 patients were analyzed for %IG on the XN series and simultaneously, a blood smear was made for automated microscopic differentiation of 500 WBC using the DM-96. Microscopic classification of the WBCs was independently performed by 2 hematology experts and mean values were used. The classification was based on the definitions published by the College of American Pathologists1. We have compared the %IG on the XN with the sum of the % promyelocytes, myelocytes and metamyelocytes counted using the DM-96.

Results: The %IG was significantly higher when analyzed by the XN analyzer compared to the microscopic differentiation (P < 0.0001; Wilcoxon test for paired samples). The mean difference in %IG between the XN and the microscopic differentiation is +2.5% (p<0.05). In figure 1, the XN %IG and microscopic %IG are compared with the optimal regression line (y=x) and corresponding confidence interval for microscopic differentiation based on 500 WBCs obtained from Rümke’s table (Rümke, NTVG 1976). Fifty-seven percent of all samples fall outside the 95% confidence interval indicating a significant difference between the %IG on XN and by microscopic differentiation. Conclusions: These results indicate that counting the immature granulocytes using the XN series shows a systematic positive error compared to manual microscopic differentiation. The percentage immature granulocytes is not interchangeable between manual microscopic differentiation and the XN series.

Introduction: Biological fluid (BF) cell count - an extremely important test for the diagnosis and follow-up of diseases—can benefit from the introduction of automated analyzers. The reference method for cell counting is the manual cytometric chamber technique, though it is slow, shows important interobserver variability, and can produce errors. Automatization of such measurements with a view to standardizing and simplifying these tests would be desirable. In previous studies we had validated body fluid mode on Sysmex XE 5000 for use in our laboratory.

Objective: Evaluation and comparison of the BF module on the new Sysmex XN-1000 versus the BF module on the Sysmex XE-5000.

Methods: 70 BF samples, [30 cerebrospinal fluid (CSF) and 40 serous fluids (10 synovial fluid and the rest were ascitic and pleural fluid)], were used for method comparison between the XN-1000 and XE-5000. We have compared the leucocytes count(WBC) and cellular differentiation (PMN and MN), red blood cells count(RBC) and high fluorescence cell count(HFC). We used for comparison the Passing–Bablok method. Results: Good agreement was found for overall with correlation coefficient r> 0.95. However, in some cases, the confidence intervals (IC) did not include the 1 value and therefore, the results and normal limits would not be transferable between methods. For WBC, r=0.997, slope=0.871(IC 0.820 to 0.934), intercept =-0.9(IC -1.8 to -0.3); PMN, r=0.921, slope=1.044(IC 0.98 to 1.092), intercept=-4.8(IC -6.79 to -1.0); MN, r=0.907, slope=1.073(IC 1.027 to 1.119), intercept=-1.07(IC -1.8 to -0.3); PMN, r=0.921, slope=1.044(IC 0.98 to 1.092), intercept=-4.8(IC -6.79 to -1.0); MN, r=0.907, slope=1.073(IC 1.027 to 1.119), intercept=-1.07(IC -1.8 to -0.3). In the HFC we found an excellent correlation also, r=0.965, slope=1.241(IC 1.0 to 1.542), intercept=0.00 (IC 0.01 to 0.01). However, the mean value for %HFC in XN 1000 was slightly higher (1.6) although with statistical significance p=0.016 (figure 1). This would yield an index of agreement (Kappa) of 0.94. In this case we would mean that if we estimate 5% of HFC as the trigger to study the cytospin, we would have studied 4(8%) fluids more, that given the pathology looked for (malignancy) is something inherently good. Conclusions: BF module XN-1000 seems to present better performance than the XE-5000 for the study of biological fluids.
130 PERFORMANCE AND EFFICIENCY OF CELLAVISION DM96 FOR REVIEW OF PERIPHERAL BLOOD SMEARS INCLUDING RED BLOOD CELL MORPHOLOGY
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Introduction: Microscopic examination of peripheral blood smears (PBS) requires review of all three blood cell types: leukocytes (WBC), erythrocytes (RBC), and platelets (PLT). While this analysis is often an essential component of laboratory investigation of clinical disease, the process can be time-intensive, even for experienced morphologists. The Cellavision DM96 (Lund, SE) is an automated slide scanner which uses image analysis to select and pre-classify WBC for review; this function is approved for clinical use in performing WBC differential counts. The DM96 also has a function for platelet estimation but currently has only limited RBC pre-classification abilities. In this study we investigate the performance and efficiency of using images captured by the DM96 to perform PBS examination, including evaluation of RBC morphology.

Methods: Wright-stained peripheral blood smears with a variety of RBC morphologies (n=137) were reviewed by two experienced laboratory technologists by both manual light microscopy and by visual inspection of the platelet estimation fields captured by the DM96. RBC shape was graded on a 4-level scale and RBC inclusions on a 3-level scale. Inter-observer (observer 1 vs. observer 2) and intra-observer (manual vs. DM96) raw agreement was analyzed. Ten additional slides representing a variety of RBC and WBC abnormalities were selected to be evaluated by 7 technologists with a range of experience (1.25-34 years, average 15.3) who had been fully trained on the use of the DM96. The technologists reviewed WBC, RBC, and PLT characteristics as they would in clinical practice both by manual light microscopy and using images captured by the DM96; times required for evaluation by manual light microscopy vs. DM96 were compared.

Results: Weighted kappa statistics for intra-and inter-observer agreement on RBC shape and inclusions ranged from 0.71 to 0.84. There were no statistically significant differences in frequencies of manual and DM disagreement of >1 grading level. Slide interpretation was on average 42.7 seconds faster using the DM96 (range -9.91). There was increased time saved using the DM96 on specimens with lower WBC (Pearson’s r=-0.8 excluding one outlier with left-shifted neutrophil leukocytosis).

Conclusions: Analysis of RBC morphology using the DM96 is reproducible between observers and comparable to analysis by conventional light microscopy. The DM96 can be used to evaluate RBC morphology, as well as WBC differential counts and morphology and platelet estimates. Analyzing PBS on the DM96 requires less technologist time than manual light microscopy, especially in cases with low WBC counts.

132 EXCESS WBC LYSIS IN THE WNR CHANNEL OF XN SERIES (SYSMEX) IS ASSOCIATED WITH METASTATIC ADENOCARCINOMA
Phuong NGUYEN, Delphine GOBIN, Rong LI, Brigitte CANTINIEAUX
CHU Saint-Pierre and Institut Jules Bordet Bruxelles, Belgium

Introduction: Background XN series are hematology analyzers newly developed by Sysmex (Kobe, Japan). The XN have three channels for WBC (White Blood Cells) counting: WNR (White cell nucleated channel), WDF (White cell differential channel) and WPC (White precursor cell channel). The WBC currently used is derived from WNR channel. Here, we report 3 cases with strong WNR-WBC underestimation. Cases description Between May and December 2013, we have observed three patients having important reproducible (different blood samplings during several days) underestimations (80 to 95%) of total WBC level counted by WNR channel compared with other channels and cytometry. The WNR scattergrams show intense debris while WDF and WPC scattergrams are completely normal. All the three patients suffered from adenocarcinoma: two with pancreatic cancer and liver metastasis treated with cisplatinum, the other with pulmonary cancer and multi-metastasis in liver, bone and ganglions without treatment. Sysmex’s hypothesis is a paraneoplastic syndrome with hypersecretion of hyaluronidase or hyaluronic acid causing a hyperlysis/hyperaggregation of WBC only with WNR reagent, the WNR counts become falsely low, the WDF and WPC channels not being affected by this phenomenon. Methods: To verify the Sysmex’s hypothesis, we added hyaluronidase (Hyalase, Wockhardt, UK) or hyaluronic acid 15000-30000Da (Sigma-Aldrich, Belgium) in the whole blood tubes of healthy patients, without any medication. The final concentrations were 30 and 75UI/mL of hyaluronidase and 2mg/mL and 6mg/mL of hyaluronic acid (n=6 for each concentration). The WBC analysis in all three channels (WNR, WDF and WPC) were assessed immediately and after 2h, 4h, 6h, 24h and 48h at room temperature or incubated at 37°C. Results: No significative difference among the WNR, WDF and WPC white cell counts in different incubation times and temperatures was observed for all the analysed samples.

Conclusions: The hypothesis of Sysmex can’t be confirmed, therefore, further investigations should be done to find the origin of the strong WNR-WBC underestimation. However, we can associate this phenomenon with metastatic adenocarcinoma observed in all our three patients. Sysmex XN users should pay attention when validating the WNR-WBC count with scattergram showing intense debris. In such cases, the users should replace the WNR result by WBC counts from WDF or WPC channels.

134 SEPSIS AFFECTS MOST ROUTINE AND CELL POPULATION DATA (CPD) OBTAINED USING THE SYSMEX XN-2000 BLOOD CELL ANALYZER: NEUTROPHIL-RELATED CPD NE-SFL AND NE-WY PROVIDE USEFUL INFORMATION FOR DETECTING SEPSIS
Sang Hyuk Park, Chan-Jeoung Park, Bo-Ra Lee, Ki-Sun Nam, Mi-Jeong Kim, Min-Young Han, Young Jin Kim, Young-Uk Cho, Seongsoo Jang
Department of Laboratory Medicine, University of Ulsan, College of Medicine and Asan Medical Center Seoul, South Korea

Introduction: The Sysmex XN-2000 analyzer can assess 36 routine and 57 cell population data (CPD) items. In this study, we evaluated these items as sepsis biomarkers. Methods: We enrolled 280 normal control (NC) and 130 sepsis patients. These patients were classified as uncomplicated or complicated sepsis. Routine and CPD items were determined, and the results were compared at between the NC and sepsis groups, uncomplicated and complicated sepsis groups, and survivors and nonsurvivors. Results: For the detection of sepsis, CPD items NE-SFL and NE-WY showed comparative AUC of 0.909 and 0.905, respectively with routine items such as hematocrit, hemoglobin, IG, RBC, RDW, lymphocyte% and neutrophil%. For the discrimination of sepsis severity, only platelet related items showed higher AUC (0.723 – 0.748) than lactic acid (0.695). For the prediction of 28-day mortality, only CV and SD
of RDW showed higher AUC (0.766 and 0.732 each) than lactic acid (0.712). **Conclusions:** Sepsis patients demonstrated significant changes in routine and CPD items, NE-SFL, NE-WY, neutrophil-related CPD data, and routine items comparatively discriminate sepsis patients; however, these values cannot efficiently discriminate sepsis severity or predict mortality.

**136 ECCIC STUDY: STUDY OF UNEXPLAINED CYTOPENIAS BY CYTOMORPHOLOGY, CYTOGENETICS AND FLOW CYTOMETRY**

Ines Rodríguez-Hernández, Alicia Castillo, José-Tomás Navarro, Jordi Juncá, Isabel Granada, Sara Vergara, Minerva Raya, Ester Viñets, Evarist Feliu, Fuensanta Millà

Hematology Department, Institut Català d’Oncologia, Hospital Germans Trias i Pujol, Institut de Recerca contra la Leucèmia Josep Carreras, Universitat Autònoma de Barcelona. Badalona, Spain

**Introduction:** Cytomorphology by light microscopy of bone marrow aspirate (BMA) and trephine is the basis of diagnosis and stratification of myelodysplastic syndromes in the WHO and FAB classifications. Cytogenetics and FISH have been considered relevant even in absence of morphologic dysplasia in the 2008 WHO edition. Recently, some studies have reported a good correlation between immunophenotype by flow cytometry (FC) in BM and cytogenetics in MDS. Moreover, it has been found a significant number of cases suggestive of MDS by FC, some of them (14%) with cytogenetic alterations and absence of myelodysplastic morphology. The aim of this study was to prospectively analyze patients with unexplained cytopenias with the three diagnostic tools. **Methods:** Adult patients referred to the Hematology outpatient clinic to study of unexplained cytopenias (hemoglobin concentration <100g/L, neutrophil count <1.0x10^9, platelet count <100x10^9) were consecutively analyzed, once nutritional defects were ruled out. Samples of BMA were sent to cytomorphology assessment by two expert cytologists, FC, cytogenetic and FISH analysis. Patients with previous hematological diagnosis were excluded. **Results:** Between March 2012 and June 2013, 46 patients were included (59% men, median age 68 years [range 32-91]). Fifteen cases had alterations in Perls stain (at least 1% of type 3 sideroblasts or ring sideroblasts), 3 out of them (6.5%) had more than 15% ring sideroblasts. Thirteen cases (28%) had phenotypic alterations (average 0.5 in Van de Loosdrecht score). Four cases (18%) presented cytogenetic alterations. One patient was diagnosed with AML, and 14 (30%) with MDS. Seven cases were suggestive of MDS only by FC, without final diagnosis of MDS (4 immune thrombocytopenia, 2 minimal dysplastic changes and 1 ferropenia). The immunophenotypic study confirmed 3 MDS with dysplastic morphology and normal cytogenetics. One case had cytogenetic aberrations as the sole altered test, with the final diagnosis of RCUD (table). Significant mean differences were found between MDS and no MDS groups in MCV (101.3FL versus 94.9, p=0.011), platelets (197x10^9 versus 116, p=0.01) and granulocyte alkaline phosphatase (GAF) (36 versus 56, p=0.042). A trend to older age was observed in MDS group (71.6 years versus 62.9, p=0.056) and lesser hemoglobin (10.6x10^9/L versus 12.0, p=0.075). **Conclusions:** BM study of patients with unexplained cytopenias was very effective to diagnose MDS. Immunophenotype study allowed the confirmation of some cases of MDS with normal karyotype. The MDS cases had increased MCV, lower platelet count, lower GAF and a tendency to older age and lower hemoglobin.

**138 HEMOGLOBIN AND CELL BLOOD COUNTS: PROGNOSTIC VALUE IN CHRONIC LYMPHOCYTIC LeUKEMIA PATIENTS AT DIAGNOSIS**

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1Hemotherapy-Hemostasis Department. CDB. Hospital Clinic. Barcelona Spain, 2Clinical Hematology Service. ICMHO. Hospital Clinic Barcelona Spain

**Introduction:** Chronic lymphocytic leukemia (CLL) is an incurable disease of heterogeneous clinical course. For over 30 years the prognostic classification of Rai and Binet discriminate risk groups based on clinical (presence of lymphadenopathy, splenomegaly and / or hepatomegaly) and analytical variables: hemoglobin (Hb > 100 g/L), lymphocyte and platelet counts (> 100 x10^9/L). Sandfel et al (Blood 2009) have been proposed that the best cutoff to the lymphocyte value is 11 x10^9/L as a prognostic factor. Our aim was to compare the main variables of the cell blood counts (CBC) and hemoglobin value that we found at diagnosis in CLL patients and correlate these values with long-term prognosis. **Methods:** We made a retrospective study of the initial CBC and prognostic implications in patients with CLL diagnosed in the last 25 years in the Hospital Clinic of Barcelona. The diagnosis of CLL was made according to the criteria published by Hallek in 2008. Patients without laboratory data to diagnosis or not correct follow-up were excluded. The primary endpoint was the overall survival (OS) and also the time to first treatment (TFT) that were determined by Kaplan Meier test. **Results:** A total of 1002 patients (57 % male and 43 % female) were identified in our database. Baseline characteristics of the patients are shown in the Table. Median age was 66 years (range, 24-98). Median overall survival was significantly shorter in the anemic patients (33 months) with respect to patients with normal hemoglobin values (119 months) (p<0.001). In addition, median OS was also shorter in the patients with thrombocytopenia (52 months) with respect to patients with normal platelet counts (119 months) (p<0.001). We did not find any differences when we compared the number of lymphocytes at diagnosis and survival (110 months for the patients with lymphocytosis vs 124 months for the patients without lymphocytosis) (p=0.63). The TFT was also significantly shorter in patients with anemia, lymphocytosis and
thrombocytopenia \((p<0.001)\). **Conclusions:** Patients with anemia and/or thrombocytopenia have a shorter OS and TFT. A total lymphocyte count has impact only on the TFT, but not on the OS and its prognostic value still needs further discussion. Despite the significant advances during the last years in new prognostic factors in CLL as immunohistochemistry, the cyto genetic or molecular biology, the initial hemoglobin value and CBC counts obtained in the hematology laboratory are still very helpful elements to consider in the prognosis of the patients.

### Table 1

<table>
<thead>
<tr>
<th>Characteristics</th>
<th></th>
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<tbody>
<tr>
<td>All patients</td>
<td>1602</td>
</tr>
<tr>
<td>Age, median years (range)</td>
<td>63 (20-80)</td>
</tr>
<tr>
<td>Gender, male (%)</td>
<td>570 (67)</td>
</tr>
<tr>
<td>WBC median ((x10^4/L))</td>
<td>38 (2-481)</td>
</tr>
<tr>
<td>Lymphocytes total count (range)</td>
<td>28 (0.5-445)</td>
</tr>
<tr>
<td>Hb (g/L), median (range)</td>
<td>135 (45-190)</td>
</tr>
<tr>
<td>Platelets ((x10^4/L), median (range)</td>
<td>195 (10.791)</td>
</tr>
<tr>
<td>Median OS months (range)</td>
<td>162 (0-471)</td>
</tr>
<tr>
<td>Median TFT months (range)</td>
<td>64 (0-471)</td>
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</table>

#### Table 2

<table>
<thead>
<tr>
<th>Variable</th>
<th>Hemoglobin ≤ 9</th>
<th>Hemoglobin &gt; 9</th>
<th>Overall survival</th>
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<tbody>
<tr>
<td>Hemoglobin ≤ 9</td>
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<td></td>
<td></td>
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<tr>
<td>≤ 100 g/L [n=30]</td>
<td>2</td>
<td>0-3</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>&gt; 100 g/L [n=30]</td>
<td>60</td>
<td>57-81</td>
<td>0.33</td>
</tr>
<tr>
<td>Median (95% CI)</td>
<td>73</td>
<td>69-77</td>
<td>0.57</td>
</tr>
<tr>
<td>P-value</td>
<td>0.001</td>
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<tr>
<td>Lymphocytes &lt;= 11</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>≤ 1.0 x10^9/L (n=77)</td>
<td>111</td>
<td>106-136</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>&gt; 1.0 x10^9/L (n=252)</td>
<td>45</td>
<td>43-84</td>
<td>0.24</td>
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<tr>
<td>Median (95% CI)</td>
<td>110</td>
<td>106-119</td>
<td>0.19</td>
</tr>
<tr>
<td>P-value</td>
<td>0.03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Platelets &lt;= 1.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ 1.0 x10^11/L (n=81)</td>
<td>8</td>
<td>0-6</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>&gt; 1.0 x10^11/L (n=63)</td>
<td>62</td>
<td>57-81</td>
<td>0.92</td>
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<tr>
<td>Median (95% CI)</td>
<td>59</td>
<td>56-61</td>
<td>0.29</td>
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<tr>
<td>P-value</td>
<td>0.001</td>
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**COMPARISON OF NUCLEATED RED BLOOD CELL COUNTS ON XE-5000 AND XN-1000 AGAINST AUTOMATED MICROSCOPY AND FLOW CYTOMETRY.**

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Ghent University Hospital Ghent Belgium

**Introduction:** Nucleated red blood cells (NRBC) are routinely counted on automated cell counters. These have overcome the drawbacks of manual microscopy methods, which are laborious, have a high imprecision and high inter-observer variability. Accurate enumeration of NRBC is important, as these interfere with automated lymphocyte counts, and hence, might overestimate lymphocyte and white blood cell (WBC) counts. Moreover, with exception of neonatal samples, appearance of NRBC as low as 1/100WBC should be considered as an indication of potential pathology and needs further investigation. **Methods:** Leftover routine samples (K$_2$-EDTA anticoagulated blood) of 100 patients were included, 78 with positive (>1/100WBC) and 22 with negative (<1/100WBC) NRBC count, as determined on the Sysmex® XE-5000. These samples were further analyzed on the Sysmex® XN-1000 cell counter, flow cytometer (FACScanto IL, BD Biosciences, US) (FACS) and automated microscope (CellaVision DM96, Sysmex®) (DM). As reference method, flow cytometry was performed on 10 000 nucleated cells. Gating on the Syto 16+ CD45- CD34- CD71+ NRBC population. DM results were used as a visual reference check (counted on 200 nucleated cells). Automatic classification (DM-unsupervised) was compared to DM results after verification by the lab technician (DM-supervised).

**Results:** Median relative and absolute NRBC count of our study population on XE-5000 were 6.05/100 WBC (range 0-1059.2) and 0.14×10E3/µL (0-78.54 10E3/ µL), respectively. Between the two reference methods, 13 samples were discrepant. Ten samples had a positive NRBC count on DM and negative on FACS, with 6 samples differing more than 1 NRBC/100WBC. Three samples were negative for DM and positive for FACS, although the difference was less than 0.5 NRBC/100WBC. Three samples had a falsely elevated NRBC count (>20 NRBC/100WBC) on XE. Compared to FACS and DM-supervised, Pearson’s correlation coefficients \((r)\) with exclusion of the latter 3 samples were 0.998 and 0.985 (XE), 0.997 and 0.986 (XN) and 0.981 and 1 (DM-unsupervised), respectively. Sensitivity for XE, XN and DM-unsupervised methods versus DM-supervised and FACS were 100%, 98-100% and 94-100%, respectively. Specificity was lower for XE (47-55%) than for XN (66-75%) and DM-unsupervised (64-90%). Positive predictive values were 68-77%(XE), 77-86%(XN) and 74-93%(DM-unsupervised); negative predictive values were 100%(XE), 97-100%(XN) and 91-100%(DM-unsupervised).

**Conclusions:** The 4 different methods show equivalent results for NRBC. However, XE-5000 is more prone to falsely elevated NRBC, and hence, falsely low WBC. To prevent false positive NRBC results with XE or XN, it remains mandatory to perform a microscopical control, which can be performed by the DM96 microscope.

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**PERFORMANCE EVALUATION OF THE INTEGRATED SLIDE PROCESSING SYSTEM SYMSX DI-60**

Yamamoto Takamasa 1, Yoko Tabe 1, Imiko Maenou 1, Rie Nakai 1, Mayumi Idei 1, Takashi Horii 1, Takashi Miida 1, Akimichi Ohsaka 1
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**Introduction:** A complete blood count (CBC) and the subsequent morphological observation of a blood smear are one of the most common clinical laboratory tests. Recently, the automated hematology analyzer XN-Series (Sysmex, Kobe, Japan) have been equipped with an integrated automated digital imaging analyzer DI-60 (Sysmex), which provides complete automation of the sample processing with an automated CBC, slide making/staining, and digital scanning with cell pre-classification. DI-60 was performed on 10 000 nucleated cells. Gating on the Syto 16+ CD45- CD34- CD71+ NRBC population. DM results were used as a visual reference check (counted on 200 nucleated cells). Automatic classification (DM-unsupervised) was compared to DM results after verification by the lab technician (DM-supervised).

**Results:** Median relative and absolute NRBC count of our study population on XE-5000 were 6.05/100 WBC (range 0-1059.2) and 0.14×10E3/µL (0-78.54 10E3/ µL), respectively. Between the two reference methods, 13 samples were discrepant. Ten samples had a positive NRBC count on DM and negative on FACS, with 6 samples differing more than 1 NRBC/100WBC. Three samples were negative for DM and positive for FACS, although the difference was less than 0.5 NRBC/100WBC. Three samples had a falsely elevated NRBC count (>20 NRBC/100WBC) on XE. Compared to FACS and DM-supervised, Pearson’s correlation coefficients \((r)\) with exclusion of the latter 3 samples were 0.998 and 0.985 (XE), 0.997 and 0.986 (XN) and 0.981 and 1 (DM-unsupervised), respectively. Sensitivity for XE, XN and DM-unsupervised methods versus DM-supervised and FACS were 100%, 98-100% and 94-100%, respectively. Specificity was lower for XE (47-55%) than for XN (66-75%) and DM-unsupervised (64-90%). Positive predictive values were 68-77%(XE), 77-86%(XN) and 74-93%(DM-unsupervised); negative predictive values were 100%(XE), 97-100%(XN) and 91-100%(DM-unsupervised).

**Conclusions:** The 4 different methods show equivalent results for NRBC. However, XE-5000 is more prone to falsely elevated NRBC, and hence, falsely low WBC. To prevent false positive NRBC results with XE or XN, it remains mandatory to perform a microscopical control, which can be performed by the DM96 microscope.
using an automated slide maker/stainer SP-10 (Sysmex) equipped in XN-Serics. A manual microscopic review was performed independently. 2. Evaluation of system efficiency: Routine analysis of a total of 2,000 blood samples was used to evaluate the processing ability of XN-Serics connected to DI-60. Results: 1. The overall analysis accuracy of pre-classification of WBC by DI-60 was 88.4%. Regarding all normal WBCs, the accuracy was 92.4%. Good correlation for all normal cell classes was observed between final results of DI-60 analysis and XN cell counter was also good except in the case of Basophils (Neutrophils: r=0.90, Basophils: r=0.43, Lymphocytes: r=0.71). Furthermore, the DI-60 system performed with high sensitivity (71 ~ 100%) and specificity (96 ~ 99%) for blasts, immature granulocytes and NRBCs. 2. The sample processing time, started from CBC counting with XN-Serics, slide making/staining by SP-10, and digital scanning with cell pre-classification on DI-60 was 38±1 minutes/ single run (×6) and 165±12 minutes / 500 CBC samples run (×4) with no sample hold up at the DI-60 (slide preparation rate 15.6%). Conclusions: Automated morphological analysis capability of the DI-60 has potential usefulness in an integrated automated hematologic analysis system. It is expected to make a significant contribution to improved efficiency of routine hematologic analysis.

144 RETICULOCYTE HB EQUIVALENT AND HYPOCHROMIC RED CELLS IN THE DETECTION OF LATENT IRON DEFICIENCY IN NON-ANEMIC PREMENOPAUSAL WOMEN

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Introduction: Iron deficiency is the most common cause of anemia in fertile women but a hemoglobin (Hb) level within the reference interval does not exclude Iron deficiency. Deficiency occurs in progressive stages, initially with a negative balance between iron stores and the requirement for erythropoiesis, leading to a progressive depletion of stores and eventually to anemia. Erythrocyte and reticulocyte extended parameters by the Sysmex XE 5000 (Sysmex Corporation, Kobe, Japan) reflect the bone marrow activity and the erythropoiesis status. The aim was to assess the reliability of the extended hemogram reporting reticulocyte Hb equivalent (RetHe) and percentage of hypochromic erythrocytes (%HypHe) in the detection of latent iron deficiency (LID), defined as iron depletion without anemia. Methods: Two hundred and fifty consecutive non-anemic women in fertile age (18-40 year, mean 33.5 y), whose analyses had been requested by general practitioners, were included in the study. Samples were analyzed for full blood count and reticulocytes within 6 hours of collection, serum ferritin and serum iron were measured. Independent samples t test was applied in order to detect statistical deviations between the groups; P<0.05 was considered statistically significant. The diagnostic performance in the discrimination between healthy females (non anemic and with serum ferritin values >20 µg/L) and those with LID (defined by Serum ferritin < 20 µg/L and Hb > 12.0 g/dL) was assessed with Receiver operating characteristic (ROC) curve analysis and concordance with Cohen’s Kappa Index. Results: One hundred and fifty three had ferritin within reference range and Hb> 12.0 g/dL; ninety seven had LID (depletion of storage iron without anemia). Ferritin area under curve (AUC) 0.981 was found to be not statistically different from those of %HypoHe (P=0.854) and RetHe (P=0.085). %HypoHe AUC 0.924, cut off 0.9 % Sensitivity 85 % Specificity 92.5 %; RetHe AUC 0.902, cut off 31.0 pg. Sensitivity 75 % Specificity 94.7 %. Applying those cut offs the agreement between ferritin and %HypoHe was κ 0.61 and 0.56 for ferritin and RetHe.

146 ERTHROCYTE AND RETICULOCYTE INDICES BY CELL-DYN SAPPHIRE IN HEMODIALYSIS PATIENTS

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Introduction: Objectives: Anemia in patients affected by chronic kidney disease is corrected by administration of erythropoiesis stimulating agents and IV iron. Reticulocyte hemoglobin content and percentages of hypochromic red cells have been proposed as accurate measures of iron status and reliable markers for monitoring therapy effectiveness in the European Best- practice guidelines. We investigated the reliability of reticulocyte hemoglobin content (MCHr) and percentages of hypochromic red cells (HPO%) reported by CELL-DYN Sapphire analyser (Abbott Diagnostics, Santa Clara, CA, USA) in predicting the response to iron administration in patients undergoing hemodialysis. Methods: Forty patients undergoing hemodialysis were studied, 17 females and 23 males (35-83 years, mean 70.3 y). The majority of patients received IV iron before enrollment and therefore the administration was interrupted 3weeks before the study. For each patient two samples were analyzed, the baseline (after three weeks not receiving iron supplements) and after receiving IV iron for four weeks. CBC including reticulocyte indices were measured within 6 hours of collection. Serum ferritin, serum iron and transferrin saturation were also analyzed. Responders were defined as patients who had an increase in hemoglobin of at least 1.0 g/dL compared with their baseline value. The differences between responders and non-responders were evaluated using the Student t-test, P<0.05 was considered significant. To identify the efficiency of the test and the optimal cut off for predicting the response to iron administration, receiver operating characteristic analysis (ROC) was performed. Results: According to the established criteria 21 patients were responders and 19 non responders. Table summarizes the data (mean and SD) at baseline for all patients subdivided into responders and non-responders to IV Iron administration.


48 Factors that Influence the Rate of Manual Validation in the Laboratory of Hematology
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Introduction: Automated validation has become a crucial strategy in the laboratory of hematology due to the dramatic increase in the daily workload during the last decade. Examination of the Peripheral Blood Smear (EPBS) is, nevertheless, necessary in many cases. The rate of EPBS can be reduced by an adequate interpretation of the graphics and histograms (AIGH) provided by the analyzer and the existence of historical data (HD) from the patients. The aim of the study was to determine the rate of manual validation in our laboratory and the impact of HD and AIGH on it. Methods: During one month a total of 70043 routine adult and pediatric samples (mean of 3045.3 daily) were run on an Advia 2120 analyzer (Siemens Diagnostics®) for a complete blood count. For each sample that needed manual validation we determined whether HD or AIGH provided or not the same information as EPBS and made it unnecessary. Results: Manual validation was required in 82.1 samples per day, which means a 2.7% of the workload. The reasons for EPBS, according to our validation criteria, were the following: leukocytosis in 3.6% (leukocytes >20000/μl), neutropenia (absolute neutrophil count <10000/μl) in 4.4%, lymphocytosis (absolute lymphocyte count >5000/μl) in 27.8%, monocytosis (absolute monocyte count >1500/μl) in 2.1%, eosinophilia (absolute eosinophil count >2000/μl) in 1.8%, LUC (Large Unstained Cells) >7% in 12.9%, Hb <8 g/dl in 4.6%, MCV >120 fl in 1.1%, MCHC >36 g/dl in 0.2%, RDW >20% in 14.9% and thrombocytopenia (platelets <100000/μl) in 26.2%. Information obtained from HD and AIGH made EPBS unnecessary in 41.3% and 11.8% of the samples respectively. In other words, HD reduced the rate of EPBS from 2.7% to 1.59% and AIGH performed by an expert hematologist reduced the rate from 2.7% to 2.38%.

ROC analysis results

<table>
<thead>
<tr>
<th>AUC</th>
<th>95% CI</th>
<th>Cutoff (pg)</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCHR</td>
<td>0.905</td>
<td>0.77-0.97</td>
<td>30.5</td>
<td>94.7</td>
</tr>
<tr>
<td>%HPO</td>
<td>0.831</td>
<td>0.67-0.93</td>
<td>4.4</td>
<td>64.7</td>
</tr>
</tbody>
</table>

Conclusions: Erythrocyte and reticulocyte indices such as %HPO and MCHR provide direct information on iron availability and on its use for hemoglobin synthesis. MCHR proved to be a sensitive predictor of response to IV iron and %HPO a specific one; both parameters may be useful to guide iron therapy in dialysis patients.

200 Evaluation of Coagulation Status in North Indian Healthy Volunteers Using Thromboelastography and Conventional Methods
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Introduction: Thromboelastography (TEG) is relatively recent assay to analyze the coagulation state of a blood sample, providing a continuous visualization of physical changes occurring during blood coagulation. There is a paucity of published literature on assessment of coagulation status using TEG in Indian population. The primary aim of the following study is to establish normal reference values for TEG in North Indian healthy volunteers and secondary aim is to compare them with conventional plasma-based routine coagulation tests and the manufacturer’s reference range. Methods: A total of 200 healthy volunteers comprised of 100 males and 100 females of age groups between 20 and 50 years, were enrolled over a period of 1 year, i.e., 2011-2012. Thromboelastometry (TEM) was performed on TEM-A automated thromboelastometer (Framar Biomedica, Rome, Italy), using whole blood non-additive (360 μl). TEG parameters analyzed were r-time, k-time, α-angle, maximal amplitude (MA). Prothrombin time (PT), activated partial thromboplastin time (aPTT) and platelet count was performed for all volunteers. The 95% reference range was calculated as (mean-1.96 standard deviation [SD]) to (mean + 1.96 SD). Results: Our reference values for 95% of 200 volunteers were r-time: 18.8-14.2 min, k-time: 0.7-7.3 min, α-angle: 27.3-72.3° and MA: 32.1-87.9 mm. Maximum clot strength was higher in women compared with men, however statistically insignificant. Overall 14.5% (29/200) of the volunteers had at least one abnormal parameter while 74% (149/200) had deranged TEG values using the manufacturer’s reference range. Statistically significant variation was seen in r-time for 88.4% (P < 0.001), for k-time, in 87.1% (P < 0.001) and for α-angle in 83.7% (P < 0.001) and for MA in 84% (P < 0.001), between the manufacturer and our reference range. Conclusions: The efficacy of classical coagulation test has been well-established; on the contrary TEG is a fairly recent assay and its utility for patient management remains to be demonstrated. We observed TEG to be oversensitive in determining coagulopathy where there is no clinical presentation. The manufacturer’s reference values may not be appropriate for different ethnicity. TEG may give an overall representation of hemostasis; however, it cannot replace the conventional coagulation tests. We recommend the determination of normal TEG values by each laboratory for their target population.
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THE ROLE OF HIGH VON WILLEBRAND FACTOR AND LOW ADAMTS13 ANTIGENS LEVELS IN THE RISK OF VENOUS THROMBOEMBOLISM
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Introduction: von Willebrand factor (VWF) has been associated with VTE in epidemiological studies and interaction between VWF-FVIII and VWF-mediated platelet adhesion has been shown to be critical for deep vein thrombosis in mouse models. ADAMTS13 cleaves VWF into less active multimers and its deficiency is associated arterial thrombosis. Because of the close relationship of ADAMTS13, VWF and FVIII in hemostasis, we aimed at investigating the effect of these proteins on VTE risk. Methods: We included consecutive patients with lower limbs deep vein thrombosis and/or pulmonary embolism, aged between 18-70 years, without medical history of malignancy, arterial thrombosis, cirrhosis, renal failure, connective tissue diseases, pregnancy or postpartum; admitted to our clinic between January 2007 and July 2011. Blood was collected at least 1 month after stopping anticoagulation and ≥ 6 months after VTE. Controls were recruited from patients friends, without history of VTE and were matched to patients by gender and age. Exclusion criteria for controls were the same as for patients. ADAMTS13 and VWF antigens were determined by commercial ELISA and FVIII activity by FVIII deficient plasma. High VWF (>150%) and FVIII (>150%) were defined by plasma levels exceeding the 88th and 94th percentiles of the control group, respectively. To define low ADAMTS13 levels we used the 10th percentile of the controls (≤0.64 μg/ml). Odds ratios (OR) and 95% confidence intervals (CI) were presented adjusted for gender and age and medians with interquartile variation (25th-75th percentiles). Results: 358 patients with a first confirmed VTE event were admitted to our clinic, of whom 282 did not participate in the study due to the exclusion criteria (n= 249) or refusal (n= 33). Therefore, we included 76 patients (53 women, 70%) with a median age of 43 years and 96 controls (66 women, 69%), with a median age of 42 years. In controls, there was a negative albeit weak correlation between ADAMTS13 levels and VWF (r = -0.213, p = 0.037) and FVIII (r = -0.251, p = 0.014). The population was dichotomized according to VWF 88th percentile of controls. Median ADAMTS13 was lower in the group with VWF >p88 compared to the group with VWF ≤p88 in patients (0.75μg/ml, 0.53-0.95μg/ml vs. 0.95μg/ml, 0.81-1.14μg/ml, p = 0.001). Next the population was categorized into subjects with VWF >p88 and ADAMTS13 ≤p10 and this association increased VTE risk when compared to the reference category (OR 4.14; 95% CI 1.03-16.71). Conclusions: High VWF and low ADAMTS13 increased VTE in our study population. An unbalance between these two interconnected proteins might have a role in the pathophysiology of VTE.

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THROMBOPHILIA SCREENING AS PART OF PREVENTIVE MEDICINE, A ONE CENTER EXPERIENCE
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Introduction: The need of screening healthy population for thrombophilia is debatable. Since 2005, a battery of screening assays has been offered as part of preventive health services at the Rambam Health Care Campus. Thrombophilia screening includes protein C global assay, assessment of prothrombin G20210A mutation and homocysteine level, which amount to a third of the cost compared to complete thrombophilia evaluation. Methods: Between 2005-2010, 15,668 subjects visited the Rambam preventive medicine center; only 910 (5.8%) of them decided to undergo thrombophilia screening. The aim of this study was to characterize the group that chose to have screening tests and to evaluate, using a questionnaire, the effect of thrombophilia screen results on patients’ health behavior. The questionnaires were distributed at least one year after receiving the test results. Results: The population that requested thrombophilia screening was older (52±9 years) and included mostly males (76%) compared to the rest of the population (48±11 years and 64% males, P=0.0001). Out of the 910 subjects, 15.2% were found to have abnormal protein C global assay results, 6% had the prothrombin G20210A mutation and 18.5% had high homocysteine levels. Seventy percent of the subjects found positive for thrombophilia, referred to a coagulation or hematology specialist after receiving the test results. More than half of them (56%) reported receiving recommendations to engage in physical activity, 43% to reduce their body weight, 40% to start anticoagulant prophylaxis during hypercoagulable states, 15% to stop smoking and 12% to stop or avoid hormonal therapy. Among the persons found to have thrombophilia, 83% reported maintaining regular physical activity compared to 69% of those without thrombophilia (P=0.022). Approximately 2/3 of the subjects who were recommended thromboprophylaxis reported following the instructions and receiving the therapy during hypercoagulable states. None of the subjects with or without thrombophilia, who filled in the questionnaire, developed any thrombotic event after receiving the test results. Interestingly, 47% of women with thrombophilic risk factors, experienced previous pregnancy complications compared to 9% in women without thrombophilic risk factors (P=0.005). Notably, 77% of the subjects found to have thrombophilia, would recommend others to undergo screening compared with 64% of the subjects in whom thrombophilia was not revealed (P=0.021). Conclusions: Personal knowledge of thrombophilia results raised patients’ awareness and influenced their health behavior aiming to reduce the risk of thrombosis. Thrombophilia screening might be an important part of preventive medicine; however further studies assessing this strategy are warranted.

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WHAT IS THE DIFFERENCE BETWEEN APTT AND PT TEST IN HIGH HEMATOCRIT? STUDY USING A HIGH HEMATOCRIT VALUE MODEL
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Introduction: For blood clotting tests of high-hematocrit blood, The Clinical and Laboratory Standards Institute (CLSI) recommends to avoid the use of commercially available, citrate-containing vacuum sampling tubes and to adjust the volume of citrate solution based on hematocrit value of each patient. We suggested the pseudo-prolongation of activated partial thromboplastin time(APTT) value is a serious issue in our previous study. However, there were not enough data regarding prothrombin time(PT) test. We therefore examined the clotting test for PT. Methods: We took blood samples from 18 volunteers...
and added increasing amounts of 3.13 % citrate solution to create a high hematocrit value model (Ht:66.2 ± 4.9). **Results:** In a high hematocrit value model at mean APTT value, CSL1 method did not induce a significant prolongation (31.3± 3.9 versus 31.2 ± 7.0 sec at baseline; p= ns), calcium chloride solution with 0.025 mol/L induced a significant prolongation (46.9± 11.3 versus 31.2 ± 7.0 sec at baseline; p<0.001). Higher concentrations of calcium chloride solution with 0.035 mol/L did not induce a significant prolongation (33.7± 6.0 versus 31.2 ± 7.0 sec at baseline; p= ns). The PT- international normalized ratio (INR) test was not significantly different between a high hematocrit value model and baseline (1.12 ± 0.11 versus 1.08± 0.27 at baseline; p= ns). In PT test, the final concentration of calcium ion of lactic acid calcium was 9.3 mol/L, in APTT test, the final concentration of calcium ion of calcium chloride solution (0.025 and 0.035 mol/L) were 8.3 and 11.6 mol/L, respectively. **Conclusions:** The reason of prolongation at the clotting tests is to contains an insufficient calcium ion 0.025 mol/L calcium chloride solution for APTT test at high hematocrit blood. Thus, Our data suggested that the final concentration of calcium ion was important for PT and APTT test at high hematocrit blood.

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**THE LUPUS ANTICOAGULANT RESULTS FROM 1100 PATIENTS ATTENDING A HIGH-RISK PREGNANCY CLINIC**

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**Introduction:** Lupus anticoagulant (LA) is clinically the most important among all antiphospholipid antibody tests. In 2009, new guidelines for LA detection were published. The objective of this retrospective cohort study was to understand the positivity of by test methods under these recommendations in patients of infertility clinic of Cheil General Hospital (CGH). **Methods:** The study group consisted of 1,100 subjects suffering from high risk of pregnancy morbidity such as unexplained consecutive spontaneous abortions before the 10th week of gestation or one or more unexplained deaths of a morphologically normal fetus at or beyond the 10th week of gestation. We measured lupus anticoagulant upon the 2 assays recommended by the International Society for Thrombosis and Haemostasis 2009 guidelines. We used activated partial thromboplastin time(aPTT) and diluted Russell viper venom time(dRVVT) integrated test for LA detection together with sensitive aPTT(silica activator & low phospholipid) reagent. **Results:** A total of 1,100 patients tested for lupus anticoagulant over a 3-year period: aged 22–46(mean: 34.6 years). In screening test, High sensitive aPTT test with Stago PTT-LA(Diagnostica Stago, Parsippany, NJ) was positive in 46.5%(511/1,100) with cut-off of patient to Normal PTT ratio of 1.2. When with the cut-off of 99th percentile of normal PTT-LA tests(37.0 sec) of CGH control group, only 9.9%(109/1,100) were positive and all the positive patients were belong to PTT-LA positive group. In PTT LA confirm test with Staclot LA(Diagnostica Stago) in screen positive 511 patients, only 7 patients(1.4%) were positive. In integrated tests with normalization ratio(cut-off≥1.2), 1.3%(14/1,100) revealed having lupus anticoagulant. All confirmed patients in any of tests, had history of unexplained consecutive spontaneous abortions before the 10th week of gestation or one or more unexplained deaths of a morphologically normal fetus at or beyond the 10th week of gestation. **Conclusions:** In high risk of pregnancy morbidity patients in infertility clinic, only 1.3–1.4% was confirmed as anti-phospholipid syndrome. The confirmatory dRVVT integrated test results showed good correlation with sensitive aPTT test. But 3 patients showed negative high sensitive aPTT screening result with 1.2 cut-off ratios, 8 patients with 99th percentile cut-off of normal, and 5 patients showed negative dRVVT screen negative results. These suggest that step by step diagnosis is not sufficient to diagnose presence of LA, for the low sensitivity of screening tests. And the confirmatory positive rate also very low in high risk patients inferring possibility of low sensitivity of integrated test.

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**EVALUATION OF A NOVEL SYSMEX, CAP-PIERCING, MULTI-WAVELENGTH, HAEMOSTASIS ANALYSER**

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**Introduction:** We assessed the performance of a prototype entry-level cap-piercing coagulation system (development code C166M) from Sysmex Corporation Japan, in parallel with their intermediate throughput CS-2100i analyser. The C166M has a relatively small footprint (760mm x 690mm x 540mm (WxDxH)), an integrated central processor unit and pneumatic system and is controlled via a 260mm colour touch panel. The new analyser, like the CS-2100i has 8 multi-wavelength reaction detector positions, and can perform clotting, chromogenic, immunoturbidimetric assays and incorporates the same user interface as the latest model CS5100. **Methods:** Performance of the new analyser was compared to a CS-2100i using reagents from Siemens Diagnostic Healthcare (Marburg, Germany) in clotting (prothrombin time [PT] using Innovin and Thromborel S, activated partial thromboplastin time [APTT] using Actin FS and FSL, Clauss fibrinogen [Fbg], Thrombin time [TT] using Test Thrombin, one-stage factor VIII [FVIII]) chromogenic (antithrombin, with BC Antithrombin III [AT-III] and INNOVANCE Antithrombin [AT]) and Immunoturbidimetric (D-dimer [DDi], with INNOVANCE D-Dimer) test systems. For FVIII assays, all samples were tested using the multi-dilution analysis (MDA) utility, to assess linearity and parallelism of the dose response curve to detect possible false results due to inhibitors or sample activation. For all other assays a single point determination was made with automatic redilution if the relative potency was outside the range of the standard curve. **Results:** The C166M methods showed good linearity and reproducible standard curves, and gave low inter-assay imprecision using commercial normal (CV = 0.48–7.7%) and pathological (CV = 0.86–6.7%) control plasmas (preparations tested 10 times on each of 5 days). With a range of clinical and normal samples (n=200 and n=30 respectively), good correlations were observed between the C166M and CS-2100i in each of the test systems (PT, APTT, Fbg, TT, FVIII, AT-III, AT and DDi; r2 = 0.947-0.999), with no clinically significant misclassification, and data points scattered closely around the line of identity. The relative rate of analysis on C166M and CS-2100i was approximately 120 and 180 prothrombin times per hour respectively. **Conclusions:** Our results demonstrated that using the C166M analyser, routine coagulation testing and specialised assays can be performed with satisfactory levels of imprecision and showing good correlation with the CS-2100i.
Introduction: Failure to report APTT results in critically ill patients can have tragic consequences. In a patient on extra corporal life support, anticoagulant therapy with unfractionated heparin could not be monitored properly because the Sysmex CS2100i analyzer used in our laboratory repeatedly reported an ‘early reaction error’ instead of an APTT value in consecutive samples. Unfortunately, the patient died of a massive brain hemorrhage. As we encounter these coagulation curve errors with some frequency, we have sought to find a way to report APTT values in such instances.

Methods: Downey et al. (Br J Haematol. 1997;98:68-73) reported on biphasic coagulation curves seen in the MDA-180 analyzer in critically ill patients. It was shown that the first part of the coagulation curve represented the formation of a complex of CRP and VLDL, triggered by the addition of calcium (Toh et al. Blood 2002;100:2522-9). The published coagulation curves on the MDA-180 were almost identical to the coagulation curves we saw on the CS2100i with the “early reaction error” and we hypothesized that a similar mechanism was responsible for the coagulation curve errors in our patient. A simple test, explained in the figure, supported this hypothesis. Results: Curve A shows a coagulation curve with a normal sigmoid shape on the CS2100i. The APTT is defined as the point in time at which turbidity has changed 50%. Curve B shows the “early reaction error” coagulation curve with a biphasic reaction pattern similar to the curves that were seen in our patient repeatedly. Curve C shows the reaction pattern in the same sample after the addition of 1 IU of heparin. Addition of heparin abolishes the change in the second phase of the curve, but does not alter the first part of the curve. This suggests that the change in the first part of the curve is independent of coagulation. We propose that in critical situations the APTT value may be inferred from the curve by optically determining the 50% change in turbidity in the second part of the curve. The estimated APTT in curve B would thus be 42 seconds (arrow). Conclusions: Coagulation curve errors of the early reaction type can be seen on Sysmex CS2100, CA1500 and CA7000 analyzers. Since the incident mentioned above, we have inferred APTT values from the second part of the curve in samples in which a coagulation independent change in turbidity was proven by the in vitro addition of heparin.

Introduction: To prevent venous thromboembolism new oral anti-coagulation medications have recently been approved as thromboprofylaxis after orthopaedic surgery. These direct anticoagulants (DOACs) comprise of Dabigatran a direct thrombin inhibitor and Rivaroxaban a direct factor Xa inhibitor. As our hospital participated in a study comparing the anticoagulation drugs Dabigatran, Rivaroxaban and Nadroprin (DARINA) in prevention of thromboembolism we established assays to monitor the effect of these drugs on our coagulation analyser Sysmex CS2100i.

Methods: Reagents suitable for measurement of a specific anticoagulants of Siemens were compared with reagents of Hyphen. To establish the influence of Coumarins with DOACs patient samples with elevated INR were used. To establish the influence of LMWH with DOACs artificial obtained samples ascending concentrations LMWH were spiked with reversed concentrations of DOACs.

Results: Analysing the within reproducibility of all assays revealed an acceptable coefficient of variation. However when analysing the in-between run, the HTI, DIxals, and HypHep performed poorly as the mandatory variation coefficient was not met. As the PT and aPTT were not sensitive enough, it was decided to quantify Dabigatran with the DTI and Rivaroxaban with the Berriv assay. Analysis of the effect of Coumarins demonstrated no influence on all assays. The presence of LMWH quantifying Rivaroxaban with the DiXals assay deviated significantly from the control levels established with Rivaroxaban alone in contrast to the Berriv test. The presence of Rivaroxaban when quantifying Dabigatran by DTI did not influence the results, however, when determining Dabigatran with the HTI and LMWH with Berhep or HypHep test there was a serious effect on outcome of these assays.

Conclusions: Overall the chromogenic assays perform better than the calibrated clotting time assays on the Sysmex CS2100i. Based on our reproducibility results LMWH could be best determined with the Berhep assay, Dabigatran with the DTI assay and Rivaroxaban with the Berriv assay. In the hypothetical case that a person enters the hospital harbouring two anticoagulants or when anticoagulants are unknown one should be aware of their influence on the respective clotting tests. Here we present evidence that using Siemens reagents one should only be concerned when switching Rivaroxaban to LMWH.
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D-DIMMER REFERENCE VALUES IN MOROCCAN PREGNANT WOMEN
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Introduction: D-dimer is a fibrin degradation product which reflects fibrinolysis and coagulation activation. In the normal population, a high rate of D-dimer evokes an acute venous thrombo-embolism (VTE). During pregnancy, a high thrombotic risk period, D-dimer testing is commonly needed to establish VTE diagnosis. Nevertheless, its contribution is hampered by the substantial increase of D-dimer rate during pregnancy. In this aim, a prospective study has been performed from December 2012 in hematology laboratory of IBN ROCHD university hospital of Casablanca with purpose to establish the reference values of d-dimer during pregnancy, especially that there is no data appropriate to our population. Methods: 86 healthy pregnant women (34 first trimester, 30 second trimester, 22 third trimester), age 18–44 years (median 28 years), were collected and investigated using monoclonal antibody-coated latex agglutination assay on ACL Top LCD controller with a normal reference value of 232 ng / ml. Only normal pregnancies were selected using the following exclusion criteria: personal or family history of thrombosis, surgery during pregnancy, neoplasia, venous insufficiency, diabetes, hypertension, smoking... Results: There was no further significant change during the first trimester, only 12% women had a high d-dimer rate with a mean of 258 ng / ml [normal reference value: 232 ng / ml]. In the second trimester, d-dimer mean was about 354 ng/ml with a high value in 72% of cases. This rate has further increased in third trimester to 517 ng/ml with only 6% women who had normal level of D-dimer. The study is still in progress fora better evaluation of results. Conclusions: Different studies underlined the progressive physiological increase of plasmatic D-dimer during pregnancy. It implies the unreliability of this test in VTE diagnosis in pregnant women. Therefore, it is necessary to determine reference values appropriate to each pregnancy trimester to help for betterand rapid diagnosis and care if venous thrombo-embolism.

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PROTHROMBIN TIME AND ACTIVATED PARTIAL THROMBoplastin Time VALUES AMONG GERiatric IN NATIONAL HOSPITAL ABUJA NIGERIA
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Introduction: Coagulation profile (prothrombin time (PT) and activated partial thromboplastin time (APTT) are screening test for Haemostasis. 40 patients consisting of male and female, undergoing surgery at National Hospital Abuja were recruited for this research work, and were screened for PT and APTT. The result shows an increase in the value PT and APTT at one hour of sample collection among the study subjects and decrease in the subsequent hours to the normal value. The result shows an increase in the male compared to the female one even though the increase was not significant, this could raise a concern of a bleeding disorder, severe deficiency of factor XII, and factor VII activity and fibrinolysis. This work is aim at determining the activity of coagulation profiles of old people who are undergoing surgery and it consequences. INTRODUCTION The prothrombin time (PT), activated partial thromboplastin time (APTT) and bleeding time (BT) are screening test for haemostasis. Typical indications for ordering these test include hemorrhagic symptoms, monitoring of anticoagulant therapy, and routine pre operative screening(Mann et al,1999). When platelets and clotting factors circulate in an inactive form, blood flows freely through the vascular system. However, vascular injury and the resulting disruption of the endothelium lead to the initiation of a complex haemostatic response broadly classified into primary and secondary haemostatic response (Davie et al,1991), bleeding symptoms in the patients or in a member of the patients family can often prompt laboratory evaluation to test for bleeding disorder. To screen for bleeding disorders and to distinguish between congenital and acquired disorder s physician should obtain a detailed personal and family (haemostatic) history and perform a through physical examination. Spontaneous bleeding (epistaxis, ease of bruising, joint bleeding) or unusual or unexpected bleeding after surgery. The PT, APTT and BT are screening test for haemostasis. The bleeding time test has lost favour in recent years, but the PT and APTT remain the most frequently ordered tests in coagulation medicine. To properly managed patient physicians must determine whether the prolonged PT, APTT and BT are artificial, medication related to representative of haemostatic abnormalities.(Arkin et al,2003). Activated Partial Thromboplastin Time. The APTT is a measure of the integrity of the intrinsic and final common pathways of the coagulation cascade. The APTT represents the time, in seconds, for patients plasma to clot after the addition of phospholipid, an intrinsic pathway activator, and calcium. The APTT reagent is called partial thromboplastin because tissue factor is not present in conjunctions with the phospholipid as it is in the PT reagents. Haemostatisis is the mechanism which involved maintenance of an intact vascular system, free from blockage in which the blood can circulate in fluid state. The normal haemostatic response to vascular damage depends on closely linked interaction between the blood vessel wall, circulating platelets and bloodcoagulants factors. The mechanism which in conjunction with inflammatory and general responses help protect the integrity of the vascular system after injury( Famodu et al,1987). The coagulant mechanism that maintains blood in fluid state it is the study of the blood to form clot and dissolve clot spontaneously to prevent occlusion of the vessels(Rodak, 1995). Haemostasis result from several interactive system designed to prevent or stop bleeding. Integral pathways of the haemostasis include blood vessel platelets, coagulation and fibrinolysis. Coagulation involves a biological amplification system, Activator substances activate the clotting cascade which culminates in fibrinogen into fibrin that enmesh the platelets aggregate at injured site and converts it to stable fibrin-platelets plug (Hoff brand et al,1995).

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AGE-BASED DIFFERENCE IN HEPARIN MONITORING IN PEDIATRIC ECMO
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Introduction: Unfractionated heparin (UH) is routinely administered for children on extracorporeal membrane oxygenation (ECMO) in order to prevent clot formation in the circuit. UH therapy is monitored by APTT and anti-Xa assay in our hospital. Although anti-Xa assay is considered as superior, hemolysis and hyperbilirubinemia which are not uncommon in pediatric patients undergoing pediatric ECMO (ECMO) therapy, APTT is a screening test for coagulation abnormalities. The APTT may be prolonged in patients requiring heparin in-Hospital ECMO therapy and is routinely measured using the International Normalised Ratio (INR) method. Our purpose is to determine if age-based differences influence the APTT in Pediatric ECMO patients.

Methods: We retrospectively evaluated 175 consecutive patients on ECMO between 2010 and 2013. We measured the APTT and anti-Xa activity in both young and old children. We compared our results to published data on pediatric patients. The APTT was measured using the activated partial thromboplastin time (aPTT) of the Stago ACL Talasys (Stago, Asnieres, France) and the anti-Xa activity measured using the anti-Xa assay (HM-4000; Diagnostica Stago, Asnieres, France). The aPTT was measured with the following reagents: aPTT reagent, aPTT Thromboplastin, and aPTT Thromboplastin blank. The anti-Xa activity was measured using the following reagents: anti-Xa reagent, anti-Xa lyophilisate, and anti-Xa blank.

Results: The APTT was significantly prolonged in older children, with a mean of 130.8 seconds (95% confidence interval [CI], 124.5 to 137.1) in newborns and 170.3 seconds (95% CI, 158.9 to 181.7) in older children. The anti-Xa activity was significantly lower in older children, with a mean of 0.91 (95% CI, 0.85 to 0.97) in newborns and 0.73 (95% CI, 0.64 to 0.82) in older children.

Conclusions: Age-based differences in the APTT and anti-Xa activity were observed in Pediatric ECMO patients. These differences may influence the interpretation of the results and the management of heparin therapy in Pediatric ECMO patients.
ECMO may interfere with anti-Xa assay. The aim of this study was to compare APTT with anti-Xa assay and UH infusion rate in order to evaluate its usefulness for UH monitoring in pediatric ECMO. Methods: Cohort study from tertiary care children’s hospital. Assays for PT, APTT, APTT-Hepzyme, antithrombin (AT), anti-Xa activity, and fibrinogen were performed on STA-R™ coagulation analyzer on day 5±2 of ECMO. UH infusion dosage was prospectively collected and analyzed using Mann-Whitney U test and Pearson correlation (as well as Spearman correlation - data not shown) (SPSS 2012). Results: There were 47 children (51% neonates, ≤1 month old) on ECMO for 7 days (4-12) who received continuous infusion of UH infusion rate of 27 units/kg/hour (22-34). AT level was 102% (84-113) and anti-Xa activity was 0.37 units/mL (0.24-0.45) with no age difference between these groups. However, neonates had significantly longer PT, APTT, APTT-Hepzyme, and lower fibrinogen compared to older pediatric patients despite the same goals (Table). There was no correlation between APTT and anti-Xa in neonates (r=0.06, p=0.78), but these variables correlated well in older children (r=0.49, p=0.02). When two outliers with APTT >150 sec were removed from neonatal group, anti-Xa correlated with APTT similar to older children (r=0.47, p=0.03). However, anti-Xa showed better correlation with APTT/APTT-Hepzyme ratio (r=0.67, p=0.0006 in neonates, r=0.63, p=0.001 in older children) than APTT alone. Of interest, APTT/APTT-Hepzyme ratio also showed good correlation with infused UH dosage in the whole neonatal group (r=0.67, p=0.0003) and older children (r=0.53, p=0.01) while anti-Xa was not (r=0.23 and 0.34 respectively, p>0.1). Conclusions: Neonatal APTT is more sensitive to UH compared to older children likely due to developmental immaturity of hemostasis. Determination of APTT/APTT-Hepzyme ratio seems to be superior over APTT alone to monitor UH therapy in pediatric ECMO settings if anti-Xa assay fails or is not routinely available.

224 HAEMOSTATIC ABNORMALITY ASSOCIATED WITH DEEP VEIN THROMBOSIS IN SUDANESE PATIENTS. Alfatih aboalbashir yousif,MSc,MT(MSc), Elwaleed Elamin,PhD, Abdelrahim Muddathir,PhD
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Introduction: 1. INTRODUCTION The functions of the hemostasis system must be strictly regulated. There is a constant balance between the factors that stimulate the activation of hemostasis and those that inhibit and control it. This means that hemostasis plays sensitive role in maintaining the balance between thrombosis and bleeding manifestations. Inhibitors prevent the coagulation process are protein C, protein S and AT III(1). Activated PC has anticoagulation properties. As a complex formed with PS, through limited proteolysis, PC inactivates coagulation factors Va and VIIla(2),free PS, is responsible for the cofactor activity. Besides F Va and F VIIla inactivation. (3). Venous thromboembolism (VTE) is a widespread clinical problem associated with significant morbidity and mortality.(4) Methods: 2. RATIONAL The incidence of deep vein thrombosis(DVT) is 1per1000person-years. 30 percent of patients die within three days; one in five suffer sudden death due to pulmonary embolism (PE)The10-year recurrenceriskis30%.Deficiencies of natural anticoagulants protein C and protein S are rare

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anticoagulant inhibitors protein C and protein S among DVT patients. Methods: Cross sectional study included 138 Patients attended outpatient clinic at Khartoum teaching hospital aged 18 or over and diagnosed as DVT data were entered and analysed using SPSS and 3 ml of Venous blood was collected in trisodium citrate. 5.1. Laboratory methods: 5.1.1. PC: PC activities were measured on SYSMEX Instrument (CA-500 model 560 Japan) using reagents (DiaMed Switzerland). The activities of PC were assayed using a chromogenic substrate method (DiaMed Protein C). According to manufacture protocol. 5.1.2. Protein S Total protein S was measured by Enzyme Linked Immunosorbant Assay using protein S Kits (AESKULIS KIT) according to manufacture protocol. Results: We measure 138 patients with mean age 48 years including 93 female 45 male venous thrombosis. Our results show that acquired risk factors are the most common case of DVT in our population. In this study 39 patients (25.5%) percent have Protein C deficiency, 17 patients (12.5%) percent have Protein S deficiency also 12 patients (8.6%) have Protein C and S deficiency. Conclusions: We conclude in comparing the results of this study with our previous study on DVT patient suggest that among inherited risk factors of DVT in our population deficiency of Protein C,S are the most prevalent factors. The results of this study could help clinicians in risk assessment and to mange their patients under diagnostic and prognostic points of view, as well as how long and how intensively to treat patients to enhance the out come.

300 CLINICAL SIGNIFICANCE OF CO-EXPRESSION OF ABERRANT ANTIGENS IN ACUTE LEUKEMIA: A RETROSPECTIVE COHORT STUDY IN MAKAH AL MUKARAMAH,
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Introduction: Aberrant phenotype in acute leukemia is reported to have variable frequency and variable relation to the disease outcome. The prognostic and predictive relevance is controversial. The aim of our study is to determine the prevalence of aberrant antigens in our cases of acute leukemia, to detect any unique clinical features at presentation that could be related to aberrant antigen expression and to correlate their expression with treatment outcome. Methods: A total of 73 newly diagnosed acute leukemia cases (40 cases of Acute Myeloid Leukemia and 33 cases of Acute Lymphoblastic Leukemia) presented to our laboratory at King Abdullah Medical City, Saudi Arabia, were included in this retrospective Cohort study. Diagnosis was based on WHO criteria and FAB classification. Also, Immunophenotyping by flow cytometry, conventional karyotyping on metaphase cells and Fluorescence in situ hybridization (FISH) for gene-fusion by Interphase were performed. Results: Aberrant antigen(s) co-expression was detected in 27 cases out of 40 (67.5%) Acute Myeloid Leukemia (AML) cases and in 14 cases out of 33 (42.4%) Acute Lymphoblastic Leukemia (ALL) cases. Out of total AML cases, CD56 expression was seen in 11 cases (27.5%) and CD7 was expressed in 7 cases (17.5%). CD7 co-expression showed statistically significant relation with unfavorable cytogenetic pattern (P= 0.046). CD4 was co-expressed in 5 AML cases (12.5%). CD19 was detected in 4 AML cases and was associated with M2 FAB phenotype and t (8; 21) in 3 of them. Out of total ALL cases, CD33 was co-expressed in 7 (21.25%) and CD13 co-expression was found in 5 cases (15.15%). There was a statistically significant higher Total Leukocyte count at first presentation for AML cases with positive Lymphoid antigen co-expression (P= 0.05). AML cases co-expressed two or more lymphoid antigens showed a lower median Relapse Free Survival (RFS). Also, There was significant higher blast count in ALL cases with aberrant antigen(s) co-expression at presentation and at day 14 of chemotherapy (P = 0.005, p= 0.046 respectively). Conclusions: Aberrant antigen(s) co-expression may be associated with adverse presenting clinical data. AML cases that co-expressed two or more lymphoid antigens had shorter median RFS. No specific cytogenetic pattern is associated with aberrant antigen expression. However larger study is recommended to get benefit of these data in clinical practice.

302 NON CLASSICAL MONOCYTES ANALYSIS IN B-CELL CHRONIC DISORDERS USING MULTIPARAMETRIC FLOW CYTOMETRY AND HEMATOFLOW™
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Introduction: Recently, a new nomenclature was published for monocytes (Blood, 2010, 116:e74-e80) which individualizes three immunophenotypic categories: classical CD14++/CD16-, intermediate CD14++/CD16+ and non classical CD14+/CD16++. If the involvement of non classical monocytes was often pointed out in sepsis, HIV infection or autoimmune disease, few publications have reported such a role in B cell disorders. Using a reference 10 color multiparametric flow cytometry (MFC) method (Beckman Coulter Navios) and the Hematoflow™ system, we studied the levels of non-classical and classical monocyte percentages in B cell chronic disorders. We also compared fluorescence intensity levels for several monocyte- and granulocyte-related monoclonal antibodies. Methods: Thirty six patients were enrolled in the study, 15 with a chronic lymphocytic leukemia (CLL) and 21 with a B-cell non Hodgkin lymphoma (B-NHL). Normal peripheral blood samples were used for reference normal monocyte subpopulation The MFC panel was CD36-FITC / CRTH2+CD2-PE / CD38-ECD / CD33-PC5.5 / CD14-PC7 / APC / CD19-APC700 / CD11b-APC750 / CD16-PE / CD45-APC / CD32-PE / CD64-ECD / CD33-PC5.5 / CD14-PC7 / APC / CD19-APC700 / CD11b-APC750 / CD16-PE / CD45-KO in a first tube and CD36-FITC / CD32-PE / CD64-ECD / CD33-PC5.5 / CD14-PC7 / APC / CD19-APC700 / CD11b-APC750 / CD16-PE / CD45-KO in a second tube. The CytoDiff™ panel of the HematoFlow™ system was CD36 FITC / CD2+CD294 PE / CD19 ECD / CD16 PC5 / CD45 PC7. A specific gating using Kaluza™ software was applied to data recorded with the MFC method and the automatic gating provided by the HematoFlow™system. For 12 patients, monocye percentages were established both by MFC and HematoFlow™, s values. Results: Higher values of non-classical monocyte percentages were observed in B-cell disorders compared to normal individuals (respectively 14.4 ± 11.5 % and 7.4 ± 3.3 % p < 0.002). Moreover, within B-cell disorders, a different partition of classical and non-classical monocytes was found between CLL and B-NHL (p < 0.05). Differential expressions of differentiation antigens on non-classical and classical monocytes were found using a specific gating with Kaluza™ software. Good correlations for classical and
non-classical monocyte percentages were found between MFC and Hematoflow™ methods (respectively r = 0.87 and r = 0.83) for the 12 samples compared. **Conclusions:** The role of non-classical monocytes remains to be evaluated and precise in B-cell disorders. A systematic approach using an automated system such as Hematoflow™ could be of interest since it is able to well individualize non classical and classical monocytes.

**304 DETECTION OF MINIMAL RESIDUAL DISEASE BY FLOW CYTOMETRY IN ACUTE LEUKEMIA: A CHALLENGE FOR STANDARDIZATION AND INTERPRETATION.**

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**Introduction:** Detection of Minimal Residual Disease (MRD) in patients with acute leukemia is important criterion for risk stratification. Flow Cytometry (FC) has been used as powerful technique in the detection of this population. However, the necessity of standardization and expertise only a few laboratories perform this study, especially in Brazil. Here, we demonstrate technical advances in the detection of MRD by FC in patients with acute leukemia, and present follow up cases to analyze remission or relapse after MRD detection. **Methods:** In 2013 we identified 50 residual population in MRD study by 5-colors FC (Cytomics FC500 and Navios, Beckman Coulter). Our strategy was based on identification of aberrant markers found at diagnosis. To collect over than one million of events, we have concentrated the cells in minimal volume plus protein, and somatization non-specific binding we have used isotype control. This study include patients with 44 median age (2 to 88 years old) who have made therapy or bone marrow transplantation and request of MRD study in our service.

**Results:** We identified 50 bone marrow samples with MRD grouped into 28 cases of Acute Myeloid Leukemia (AML), and 22 of B lymphoblastic leukemia (B-ALL). 64.3% (18/28) of AML patients still had MRD after therapy and/or transplantation. 25.0% (7/28) had complete remission, 10.7% (3/28) of cases, our MRD detection predict relapse. Among the 36 B-ALL, 4% (8/22) had MRD after therapy and/or transplantation, and 59.1% (13/22) had remission. Only 1 case (4.6%) relapsed after therapy with MRD(-) during treatment. Among the techniques used to MRD study, FC is highlighted due to the sensibility of 0.01% and could be used for risk stratification in acute leukemia. **Conclusions:** Our focus was to detect the aberrant phenotype in immature cells. B-ALL is more established than AML due to knowledge about B-cell maturation, and the different expression of CD38 and CD58 in normal or neoplastic immature B-cells. Heterogeneity is hallmark of AML, so MRD by FC is a challenge, mainly in interpretation. The strategy used to evaluate the efficiency of our MRD detection was to follow up the patients and monitor the remission or relapse. Briefly, 8.0% (4/50) of cases, our technique could predict relapse, and probably, if the follow up were not so short, this number could be higher. Therefore, our group have been demonstrated the ability in monitoring the residual population in acute leukemia, despite all limitations in standardization, monitoring and interpretation of MRD analyses.
FINE NEEDLE ASPIRATION AS A DIAGNOSTIC TECHNIQUE IN B-CELL NON-HODGKIN LYMPHOMA: THE USEFULNESS OF COMBINING CYTOMORPHOLOGY WITH FLOW CYTOMETRIC IMMUNOPHENOTYPING AND FISH ANALYSIS

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Introduction: The role of fine-needle aspiration biopsy (FNAB) in the primary diagnosis and subclassification of Non Hodgkin lymphoma (NHL) has been under intensive debate for a long time. The World Health Organization (WHO) classification of tumors of the hematopoietic and lymphoid tissues 2008 is based on their cytomorphologic, immunophenotypic, and genetic features. These criteria can be readily applied to cytologic specimens. This study was designed to evaluate the value of lymph node FNA specimens in the diagnosis and subclassification of NHL according to WHO classification 2008 focusing primarily on B-NHL. Methods: The current study was prospectively conducted on 129 patients with lymphadenopathy and a clinical suspicion of lymphoproliferative disorder. The sampling procedure was performed using fine needle sampling without aspiration. Cytomorphologic examination was performed on Leishman stained smears. Representative material for cytologic examination was available for only 120 cases (93.02%). One hundred-twenty two (94.57%) specimens collected on RPMI-1640 were submitted for flowcytometric immunophenotyping (FCI) using a battery of monoclonal antibodies specific for lymphoid antigens. I-FISH analysis was performed on 20 out of 48 cases diagnosed as B-NHL (by cytomorphology and FCI) for chromosomal rearrangements t(11;14)(q13;q32) and/or t(14;18) (q32;q21). Results: Using a multiparametric algorithm including clinical data, cytomorphology, FCI, and FISH analysis, definitive final diagnosis was reached in 116 (90%) cases (table 1). Table (1): Distribution of cases according to the final diagnosis

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Number (116)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benign</td>
<td>45</td>
</tr>
<tr>
<td>B-NHL</td>
<td>48</td>
</tr>
<tr>
<td>T-NHL</td>
<td>7</td>
</tr>
<tr>
<td>Hodgkin lymphoma</td>
<td>8</td>
</tr>
<tr>
<td>Others</td>
<td>8</td>
</tr>
</tbody>
</table>

Forty eight cases had a final diagnosis of B-NHL. Of these, 33 cases (33/48, 68.8%) could be subclassified according to WHO classification 2008 (figure 1). For the sake of validation studies of FNA as a diagnostic method, a gold standard was established to control FNA diagnoses. This comprised histopathologic examination, FISH, clinical control (LDH, β2-microglobulin, and imaging studies), and follow-up of the clinical course (for at least 6 months), or a combination of them. The estimated diagnostic sensitivity of FNA combined with ancillary studies in distinguishing between reactive and malignant lymphoid proliferations was 98.39%, with 100% specificity and 98.89% diagnostic accuracy. Sensitivity, specificity, and diagnostic accuracy of the FNA in B-NHL diagnosis were 98.04%, 100%, and 98.89% respectively. Conclusions: Lymph node FNA offers a simple, safe, and minimally invasive procedure for obtaining a diagnostic material that allows a rapid accurate diagnosis and subclassification of B-NHL through the integration of cytomorphology, FCI, and FISH analysis.
DEVELOPING A FLOW CYTOMETRIC METHOD FOR DETERMINING THE MUTATIONAL STATUS OF NUCLEOPHOSMIN (NPM1) IN ACUTE MYELOID LEUKAEMIA (AML)
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Introduction: NPM1 is a shuttling protein with predominant nucleolar localisation that plays multiple roles in cell growth and proliferation. Mutations of NPM1 seem to disrupt its normal physiologic role as a molecular chaperone, which likely leads to its oncogenic potential. NPM1 if present in isolation is associated with significantly better overall survival and disease free survival in AML and has been entered as a provisional category in the World Health Organisation (2008) classification of Acute Myeloid Leukaemia with recurrent genetic abnormalities. Current methodology uses PCR techniques to detect NPM1 mutations. Although these methods are robust and relatively easy to perform they can be expensive, labour intensive and not universally available. Flow cytometry may be an alternative method for detecting the mutation at translational level. Methods: A commercially available NPM1 mutation specific antibody was used to develop a diagnostic method by flow cytometry. The antibody is polyclonal and unconjugated. A secondary antibody was used to detect the primary antibody binding. Cells were first labeled on the surface with CD45 PerCP for the purpose of gating. Red cell lysis and permeabilisation was done using FACS LYSE and PERM BD (Becton-Dickinson). Optimal antibody concentration, blocking technique for non-specific binding as well as intracellular staining methodology was established. Flow cytometry was performed using a BD FACSCanto II flow cytometer and gating was performed on CD45 expression and side scatter. The technique was tested on a commercial cell line, OCI/AML3, with a known Type A NPM1 mutation. The NPM1 mutation was confirmed on PCR. The minimum detectable concentration of NPM1 mutated cells was determined by doing dilution studies with normal peripheral blood. Samples from 12 newly diagnosed AML patients were used to correlate the NPM1 flow cytometry assay with DNA based PCR. Analysis was performed using Kaluza™ software. Median Fluorescence Intensity ratio (patient : negative control) of greater than 1.7 was established to optimally differentiate positive and negative patients on flow cytometry. Results: The flow cytometric technique effectively detected the NPM1 mutation in the positive cell line with a sensitivity of just less than 10%. 2 of the 12 patients were positive for NPM1 on both flow and PCR and 10 were negative. Conclusions: Flow cytometry may be utilised as a relatively cost effective, rapid alternative to PCR to test for NPM1 mutations and may be applicable to laboratories that do not have access to molecular services however continued validation of this technique is required.

VALIDATION OF THE CYTOGNOS SMALL SAMPLE TUBE (SST) FOR FLOW CYTOMETRIC EVALUATION OF PAUCICELLULAR CLINICAL SPECIMENS
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Introduction: A number of specimens sent to the Clinical Flow Cytometry (FC) laboratory for determination of the presence or absence of a malignant haematological cell population are paucicellular. Typical specimen types include FNA, CSF, skin and core tissue biopsies. Inadequate cell numbers can limit the diagnostic utility of FC despite an apparent abnormal cell population noted on morphology. Cytognos have developed the Small Sample Tube (SST) with 11 conjugated antibodies designed for the detection of normal and aberrant lymphocyte populations of B/T/NK and Plasma Cell lineage. Their 8-color tube contains the full combination of antibodies described in the EuroFlow SST, namely: CD3/CD4/CD8/CD14/CD19/CD20/CD38/CD45/CD56 and surface Kappa/Lambda. We validated this new tube to enable routine use in our clinical FC laboratory. Methods: We performed a validation of the Cytognos SST panel on our FACScanto II flow cytometer including voltage/compensation optimisation, accuracy assessment using BD Multi-check Control Cells and precision of each marker using a normal patient sample. Correlation/comparison with existing method was performed, involving testing on routine samples using the Cytognos SST panel and correlating the results with our existing validated “in-house” 8-colour leukaemia/lymphoma tube’s. Results: Accuracy was demonstrated for all 11 markers using Multi-check control (percentage positivity within acceptance limits across testing on 3 consecutive days) Within-run precision on 5 replicates of normal patient sample for all markers was excellent (CV<5%). For the correlation study (n=24), sample types tested were LN Bx (n=9), PB (n=6), BMA (n=4) FNA (n=1), CSF (n=1) and other fluid (n=3). Cases included 12 normal samples, 8 clonal B cell samples, 2 T-NHL samples and 2 Plasma Cell Dyscrasia samples. The comparison study showed 100% diagnostic concordance, ie. all normals using our routine panels were normal using the Cytognos SST panel while all abnormalities demonstrated using our routine panels were abnormal with similar phenotype using the Cytognos SST panel. The FC were concordant with morphological assessments. Conclusions: Validation of the Cytognos SST tube demonstrates acceptable precision and accuracy whilst excellent correlation with previous “in-house” panels has been shown. It provides broad coverage of B/T/NK lymphoid cell populations including plasma cells. It is a useful commercial cocktail for paucicellular specimens and could be used as an initial “screening” tube. It has the advantage of a single antibody aliquoting step, based on the integration of all 11 markers in the cocktail tube, providing scope for reduction in laboratory aliquoting errors.

VALIDATION OF AGE-RELATED REFERENCE RANGES FOR SERUM THYMIDINE KINASE ACTIVITY IN CHRONIC LYMPHOCYTIC LEUKAEMIA AND ITS COMPARISON TO OTHER PROGNOSTIC MARKERS
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Introduction: To date no age-related reference ranges are available for serum thymidine kinase (TK1) activity. Being a proliferation marker, it may be used as a prognostic marker in malignant diseases, including chronic lymphocytic leukemia (CLL). Our aim was to establish age-specific reference ranges for TK1 and examine its utility as a screening marker in CLL, a disease of the elderly. Methods: SerumTK1 activity was measured by a competitive chemiluminescent immunoassay in 369 healthy adults and 115 de novo CLL patients. Flow cytometric measurements were carried out on FACScanto II flow cytometer, the results were evaluated by FACSDiva software. Besides a 3-tube and 8-color CLL panel
Zap70 expression was also evaluated. The patient was considered CD38 and/or Zap70 positive if ≥20% of the CLL cells expressed the respective markers. Results: We observed a statistically significant decline in TK1 activity from young (18-35 years) to middle-aged (36-60 years) and further on to elderly (60-86 years) among healthy individuals. Age-related reference range was: <30 U/L for young, <25 U/L for middle-aged and <19 U/L for elderly. There was no difference in TK1 activity between the studied healthy men and women. In CLL patients, TK1 activity was the highest in the advanced Rai stages. The area under the receiver operating characteristic curve (ROC-AUC) for TK1 was 0.840 (95% CI: 0.787-0.892), for differentiating CLL patients from age and sex matched healthy controls, with a cut-off value of 10.5 U/L (sensitivity: 80.9%, specificity: 73.4%). TK1 was significantly elevated in CD38+/Zap70+ CLL patients, and showed significant correlation with leukocyte and absolute B-cell count. Conclusions: In healthy people, serum TK1 activity does not differ in the two sexes but declines significantly with age. As such, use of age-related reference range is warranted, especially when evaluating CLL patients who generally belong to the elderly.

316 FLOW CYTOMETRIC DETECTION OF RARE EVENTS IN MYELODYSPLASTIC SYNDROMES

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Introduction: Myelodysplastic syndromes are heterogeneous clonal hematopoietic stem cell disorders characterized by variable clinical outcome, therefore precise diagnosis and classification of cases to the appropriate prognostic category are indispensable. Flow cytometry has substantial role in the objective evaluation of these cases. Our aim was to explore this potential in our daily practice. Methods: We classified the patients to control (n=14) and MDS (n=55) groups on the basis of morphological, cytogenetic, and flow cytometric examination of their bone marrow samples. We evaluated four parameters suggested by Ogata et al. by flow cytometry. Furthermore, we investigated the percentage and surface antigen expression patterns of erythrocytes and rare events - erythroblast, mast cell (MC) and plasma cells. In the MDS group seven patients had acceptable bone marrow biopsies. We performed Wright-Giemsa-stain, silver-staining, CD117 labeling as well as tryptase immunohistochemical staining in order to ensure the detailed examination of MC. Results: When we compared MDS cases to normal bone marrows we detected significant differences in the myeloblasts (%) (1.0% vs. 0.72%, p=0.0037), in granulocytes SSC/lymphocytes SSC (6.9±1.9 vs. 7.7±3.8, p=0.0162) and in the proportion of lymphoblasts among CD34 positive cells (2.4% vs. 5.4%, p=0.04). In addition, we found significant differences in the proportion of erythrocytes (18.1±9% vs 11.3±2.6%, p=0.0001) and—in terms of rare events—that of MC (0.029% vs 0.009%, p=0.01). The ratio of plasma cells and normoblasts did not differ significantly. As for the forward and side scatter of MC and the intensity of CD71, CD117, CD45 marker expression on their surface, there were no significant differences between the two groups. In the morphological and immunohistochemical tests the MC were scattered individually, their proportion was increased in MDS patients compared to the control group (n=4), and their atypical morphology was also observed in MDS patients. An association between MC and fibrosis has been found in various disorders, therefore we investigated the fibrosis grade of crista biopsy samples, which indicated a higher mean MC ratio by myelofibrosis grade 0/1 compared to grade 2 cases. Conclusions: Besides identifying the usual flow cytometric alterations previously described for MDS, we detected an increased MC ratio, which might have a role in the development of fibrosis in some MDS cases.

318 THE ANALYSIS OF MYELOID-DERIVED SUPPRESSOR CELLS IN CANCER PATIENTS WITH NEUTROPENIA TO EVALUATE THE EFFECT OF GRANULOCYTE COLONY STIMULATION FACTOR

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Introduction: Myeloid-derived suppressor cells (MDSCs) are a heterogeneous cell population associated with immunosuppression and inflammation in cancer patients. Granulocyte colony stimulating factor(G-CSF) is frequently used in cancer patients with neutropenia. To evaluate the effect of G-CSF on patients being chemotherapy we analyzed MDSCs in the peripheral blood. Methods: Peripheral blood was collected from 10 patients accompanied neutropenia during chemotherapy before and after G-CSF administration. Flow cytometry was used to examine the percentage of MDSCs. CD45-positive mononuclear cells were gated and then CD11b-positive and HLA-DR-negative cells were considered for MDSCs. The changes of MDSCs in the peripheral blood according to G-CSF infusion were analyzed. Results: The absolute neutrophil counts in patients before G-CSF administration were 547±391 cells/ul (50-1,120 cells/ul). MDSCs were detected in the peripheral mononuclear cells with a cell percentage of 3.2±4.5% (0.2-12.2%) and 6.2±9.7% (0.2-26.9%) before and after G-CSF infusion, respectively. Elevated levels of MDSCs in 6 patients, decreased levels in 3 patients and same level in 1 patient were observed. Conclusions: G-CSF generally seems to move the MDSCs from the bone marrow to the peripheral blood to increase MDS in the peripheral blood. It should be studied that how the increased MDSCs affect the patient’s prognosis.

320 PERIPHERAL BLOOD PROGENITOR MONONUCLEAR CELL COUNT IN Apheresis PRODUCt BY TWO METHODS: PRELIMINARY DATA

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Introduction: Mononuclear cell (MNC) count represents the best control of the apheresis product (AP) in the setting of hematopoietic stem cell transplantation. However, little has been reported about the most suitable method for its determination. AIM: To study whether MNC count by optic microscopy (OM) is different by the one obtained by flow cytometry (FC), taking the latter as the reference method. To evaluate if there exist differences between the utilization of CD14 and CD45 in monocytes determination of the AP by FC. To check whether there is a relation between the MNC
count, tested by both methods, and the dose of CD34+ cells in the
AP. Methods: 17 samples of AP were smeared and stained with
Panoptic stain to count the MNC by OM. At the same time, the
samples were incubated with the following monoclonal antibodies:
CD16 FITC/CD34 PE/CD45 PercP/CD14 APC, and acquired in a
flow cytometer FACS CALIBUR. Analysis was performed with the
Paint-a-gate program. The statistical analysis was performed with
the nonparametric test U Mann Whitney for not related samples,
the Bland and Altman test and Pearson correlation calculation and
its estimation by 95% confidence intervals. Results: The MNC
count was different between both methods, with an overestimation
by OM (p=.001). These differences were determined mainly by the
monocytes count (p=.002), not being present in the lymphocytes
count (p=.670) (Figure 1). The average difference found between
monocytes by CD14 was of -3.8% with respect to CD45. 53% of
the time, there was a perfect match between both measurements.
There was no evidence of relationship between the total MNC
count by FC and the total dose of CD34 in the AP (r = -0.15,
p=.54), either when it was evaluated by OM (r = -0.19, p=.47).
We can say that the relationship between CD34 and MNC is not
strong (IC = [-0.6;0.035]) Conclusions: MNC count of the
apheresis product by OM is not a suitable method to evaluate the
quality of the product. In this sense, the cytometry represents the
tool of choice. Monocyte count with CD45, without the use of the
monocyte marker CD14, saves in the costs of the study, as it would
be possible to determine progenitor cells and MNC just with CD34
and CD45. It does not seem to be a strong relationship between
the dose of CD34 and the percentage of MNC.
**Introduction:** The transcription factor NFκB has been demonstrated to be constitutively activated in many haematological malignancies, including multiple myeloma, acute myeloid leukaemia, acute lymphoblastic leukaemia, chronic myeloid leukaemia and chronic lymphocytic leukaemia. Published data highlights the therapeutic and prognostic potential of NFκB expression in some malignancies. Studies of NFκB expression/activation are primarily performed by fluorescence microscopy, western blotting and enzymatic detection methods, all of which are useful but lack the quantitative and analytical power of flow cytometry. Antibodies specific to the phosphorylated form of NFκB are available and studies have been performed which show their applicability in the research setting; however they are not routinely used clinically. Imaging flow cytometry is a powerful platform that couples microscopy with flow cytometry, enabling quantitative cell population analysis while at the same time tracking sub-cellular localisation of intracellular markers. This has the potential to allow the translocation of activated NFκB from the nucleus to the cytoplasm to be visualised whilst simultaneously performing leukocyte immunophenotyping. It also has the potential to study the interaction of NFκB with other signalling molecules that are important in haematological malignancy.

**Methods:** We optimised an 8-colour flow cytometry panel to observe NFκB expression and sub-cellular localisation in lymphocyte populations. The panel includes CD3, CD5, CD10, CD19, CD20, CD45, nuclear stain (Hoechst) and NFκB. Our gating strategy is modelled on the PathWest diagnostic lymphoproliferative panel which first gates lymphocytes on a CD45 vs SS scatter plot, then plots for expression of CD3, CD5, CD10, CD19 and CD20. Lymphocyte populations are then analysed for expression and localisation of NFκB.

**Results:** This panel allows quantitative analysis of NFκB expression and sub-cellular localisation in large numbers of cells and in lymphocyte sub-populations. Quantitative flow cytometry data is validated against routine analysis of samples performed by PathWest on a FACSCantoII and nuclear translocation of NFκB is verified using unstimulated and RANKL-stimulated RAW264.7 mouse macrophage cells. **Conclusions:** This method can be applied to the systematic analysis of NFκB expression and distribution within cells in lymphoproliferative diseases, to identify disease entities and cell populations that have aberrant activation of NFκB.

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**FLOW CYTOMETRY OF BONE MARROW ASPARITES IN THE DIAGNOSIS OF MYELODYSPLASTIC SYNDROME**

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**Introduction:** Myelodysplastic syndrome (MDS) is a clonal stem cell disorder resulting in peripheral cytopenias including anaemia, thrombocytopenia and/or neutropenia. MDS is associated with an increased risk of developing acute myeloid leukaemia (AML). Currently, the diagnosis is based on assessing dysplasia by morphology. However, his method is subjective and subject to inter-observer variability. Recently, scores based on flow cytometry have been advocated to be a more objective tool in diagnosis MDS. **Methods:** A bone marrow aspirate was performed in 206 consecutive patients with a cytopenia. Flow cytometry was used to search for aberrancies matching MDS. Four cardinal parameters were used to screen for MDS: the percentage of precursor B cells of all CD34 positive cells, the side scatter ratio between granulocytes and lymphocytes, the CD45 ratio between myeloblasts and lymphocytes and the percentage of myeloblasts. To increase the sensitivity and specificity three additional parameters were added: the expression of CD11b, CD56 and CD15 on myeloblasts. Based on these parameters the total sensitivity and specificity were calculated. Subgroups within the MDS group (n=78) and the pathological controls (n=128) were compared to see whether there was a pattern in marker expression. Based on the total flow score we sought to see whether a high flow score correlated with a higher chance of finding MDS. In addition, the value of CD7 expression on myeloblasts was evaluated in a sub-sample. **Results:** The cardinal parameters provided a low sensitivity and specificity in detecting MDS. Adding the other parameters increased the sensitivity and specificity to 74% and 71% respectively. However, flow cytometry demonstrated a clear distinction between low grade and high grade MDS (p<0.05). It showed little difference between low grade MDS and normal bone marrows and no significantly different markers were found between high grade MDS and acute myeloid leukaemia (AML) (all p>0.1). A high total flow score did not result in a significant higher chance of finding MDS. CD7 did not significantly differ between any of the subgroups and even resulted in a decline of sensitivity and specificity for diagnosis MDS. **Conclusions:** Flow cytometry is an objective tool to aid in diagnosis MDS with an acceptable sensitivity and specificity within a population of a large non-academic hospital. However the total flow score is an unreliable diagnostic tool. Flow cytometry shows the heterogeneity of MDS as a clinical entity and should therefore be investigated for its value in risk management.
using Cytodiffr™ on FC500 or Navios flow cytometers (Beckman Coulter). Results: From 2011-2013, 67 samples were analyzed in at least 6 laboratories simultaneously, resulting in 402 analyses. The gold standard per population for each sample was the mean of all participants. Values outside +/- 2SD of that mean were considered outliers and excluded. Table 1 presents the number of exclusions and analyses performed, mean percentage and variation coefficients (CV%) of the most important populations.

<table>
<thead>
<tr>
<th>Mature neutrophils</th>
<th>No. of exclusions: 19</th>
<th>No. of analysis: 383</th>
<th>Mean %: 56.9</th>
<th>Mean CV%: 2.96</th>
</tr>
</thead>
<tbody>
<tr>
<td>B-lymphocytes</td>
<td>19</td>
<td>383</td>
<td>3.66</td>
<td>9.44</td>
</tr>
<tr>
<td>T and NK lymphocytes</td>
<td>18</td>
<td>384</td>
<td>26.98</td>
<td>3.96</td>
</tr>
<tr>
<td>Monocytes</td>
<td>18</td>
<td>384</td>
<td>7.64</td>
<td>5.41</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>12</td>
<td>390</td>
<td>3.11</td>
<td>18.06</td>
</tr>
<tr>
<td>Basophils</td>
<td>9</td>
<td>393</td>
<td>0.92</td>
<td>25.77</td>
</tr>
</tbody>
</table>

In general, Cytodiffr™ results between laboratories were comparable. The EQC enabled users to detect issues with particular flow cytometric settings or pre-analytical conditions. Cell populations creating the most disparities between laboratories were eosinophils and immature granulocytes (Mean: 0.48%, CV: 60.71%). Visual inspection of flow plots revealed this was due to misalignments by the automated software. Conclusions: The SKML hemocytometry EQC for Cytodiffr™proves a valuable tool for comparing results between laboratories. In some cases, systematic deviations are found for a laboratory, prompting further attention and/or adjustment of analyzer settings. Unfortunately samples from SKML do not contain abnormal leukocytes (blast or progenitors), which can be measured using Cytodiffr™, making it difficult to assess the accuracy of Cytodiffr™ when participating in this particular survey. Comparing results between participants provides a platform for discussing analytical and logistical aspects.

400 MOLECULAR CHARACTERIZATION OF B-THALASSEMIAS IN EGYPT

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Introduction: Thalassemia is the world’s most common monogenic disorder. About 1.5% of the global population were carriers of beta thalassemia, and 1,000/1.5 million per year live births will suffer from thalassemia disease in Egypt. To date, there are over 200 known mutations in Beta Globin Gene found to be associated with thalassemia. Methods: 200 blood samples collected from Egyptian beta-thalassemia patients were subjected to DNA isolation and molecular characterization using PCR–ARMS techniques. The study included estimation of the MCV, HbA2, HbF and transferrin saturation which was important to differentiate between iron deficiency and thalassemia carriers. Results: Twenty-six mutations are up to date identified by our working team. The five most common mutations were IVS-1 nt 110 (24.5%), IVS-1 nt 6 (19.75%), IVS-1 nt 1 (9.25%) and IVS-2 745, 848 3%, where, -87 very rare (2%) were characterized as sickle cell anemia 1.75%. Conclusions: Characterized Egyptian alleles are all Mediterranean in origin except IVS II-848, which is considered an Egyptian mutation. Further mutations characterization and genetic modifiers studies are needed for the clarification of phenotypic heterogeneity.

404 IMPACT OF WILMS TUMOR 1 (WT1)GENE MUTATIONS IN-PATIENT WITH CYTOGENETICALLY NORMAL ACUTE MYELOID LEUKEMIA

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Introduction: Wilms’ tumor 1 (WT1) mutations in cytogenetically normal acute myeloid leukemia (CN-AML) among Egyptian patients. Methods: Exons 1, 2, 3, 7, 8, 9 of WT1 were screened for mutations in samples from 82 CN-AML patients out of 203 newly diagnosed AML patients, age range from 21-74 years, using a high-resolution capillary electrophoresis technique. Results: Eleven patients out of 82 (13.41%) harbored WT1 mutations. Mutations were detected in exon 7 (n = 7), exon 9 (n = 2), exon 8 (n = 1), exon 3 (n = 1), but was not detected in exons 1, 2. There was no statistically significant difference between the WT1 mutants and wild types with regard age, sex,
French-American-British subtypes and the prevalence of success of induction remission therapy (P = 0.966; 28.6% vs 29.3%). Patients with WTI mutations had overall survival (OS) lower than the wild type one (HR = 1.38; 95% CI, 4.79 to 8.68; P = 0.004). Conclusion: Cytogenetically normal acute myeloid leukemia patients with WTI mutations have poor clinical outcome. We recommend molecular testing for WT1 mutations in with CN-AML at diagnosis in order to improve risk stratification of those patients.

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**STUDY OF FEW CYTOKINES GENES POLYMORPHISM AND EVOLUTION AFTER HSCT**

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**Introduction:** Introduction: Cytokines gene polymorphism is part of transplant immunology with strong implication in evolution and development of early and late complication. This remain an open and acute problem for study and discussion. **Methods:** Methods: We studied a lot of eighteen pairs, patients with acute leukemia, lymphoblastic and non-lymphoblastic, and their donors, for polymorphism genes of few cytokines. All related donors and recipients had 100% HLA alleles match. The source of HSCT was PBSC. The following cytokines are observed: IL-1α pos 889, IL-1α pos mspa 11100, gPNF pos 874, TFβ1 codon 10, TNFα pos 308, IL-6 pos 174, IL-10 pos 1082, IL-10 pos 592. The method used was PCR-SSP (Dynal Genotyping SSP Kit). The complications like graft versus host disease acute and chronic, relapse, TMA and the recovery with thrombocytes are followed. **Results:** Results: IL-1α pos 889, absence of CC/TC, TNF-α pos 308 GA/GA, absence of GG/GG, IL-10 pos 1082 AA/AA are favorable for early recovery with thrombocytes(<17 days) with statistical significance. gPNF pos 874, absence of AT/AT are protective again TMA. IL-10 pos 592 AA/CA are also favorable for thrombocytes recovery. No influence, with statistical significance, was established in our study. **Conclusions:** Conclusions: A study like this, a small number of pairs, genoidentical, with PBSC like HSCT source, prove us that some alleles can influence the complication like TMA, and early thrombocytes recovery.

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**BRAF V600E : SINGLE MUTATION KEY TO HAIRY CELL LEUKAEMIA STANDARDIZATION OF ALLELE SPECIFIC PCR FOR DETECTION OF BRAF V600E IN A TERTIARY CARE HAEMATOLOGY CENTER IN INDIA**

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**Introduction:** Classical Hairy cell Leukaemia (HCL) is a rare indolent B-cell chronic lymphoproliferative disorder characterized by splenomegaly, pancytopenia, presence of hairy cells in peripheral blood/bone marrow. HCL-v encompasses cases of B-chronic lymphoproliferative disorders that resemble classical HCL but exhibit variant cytogenetic features, variant immunophenotype and lack of dramatic response to purine analogues. We present here 4 cases of HCL diagnosed and treated in Tata Medical Center from May 2011-May 2013. **Methods:** 4 patients presenting with cytopenias and splenomegaly, their CBCs, differential counts, peripheral smear, bone marrow aspirates and biopsies were studied. Annexin A1 and DBA.44 immunohistochemical stains were done on BM biopsies. Flowcytometry was performed on bone marrow using CLPD panel on BD FACS Canto II. Allele specific PCR for detection of BRAF V600E mutation was standardized by extracting genomic DNA (Formalin fixed paraffin embedded tissue extraction kit, Qiagen) from the archived unstained bone marrow aspirate slides of all these 4 patients. The assay consists of 2 PCRs sharing the same reverse primer but differing for the forward primers. One is complementary to the wild type and the other to the mutated base causing the V600E replacement. After mutant AS-PCR, bands were detected on agarose gel electrophoresis. Cross verification and validation of AS-PCR results was done by separate IVD approved Real Time PCR assay. **Results:** The peripheral smear, BM aspirates and biopsies of all 4 patients displayed classical hairy cells. The hairy cells were positive for Annexin A1 and DBA.44 except for one which was negative for Annexin A1. Flow cytometric analyses exhibited characteristic HCL immunophenotype (CD20/CD22/CD11c/CD103/FMC7/CD25 positive; CD5/CD10/CD23 negative) in 3 cases except one which showed CD25 negative. So, a diagnosis of HCL-variant was suggested in this case though the hairy cells showed classical morphology. All three cases of classical HCL showed mutation BRAF V600E while the HCL-v did not. A fifth case diagnosed HCL and received 2 months of Cladirabine elsewhere was confirmed by detection of BRAF V600E mutation on PB. Immunohistochemical staining for DBA.44 on his BM biopsy showed residual interstitial hairy cell infiltrates post 3 months of Cladirabine. **Conclusions:** Allele specific PCR is a simple, sensitive, reliable and a doable assay confirming the constant presence of BRAF V600E mutation, a signature molecular marker of classical HCL as it is absent in HCL-v and HCL like disorders. It is important to diagnose HCL-v as it is a more aggressive disease and shows resistance to conventional HCL therapy. Immunostaining for DBA.44 and BRAF V600E are extremely useful to monitor the response to Cladirabine in HCLs.
A COMPARISON OF TWO POLYMERASE CHAIN REACTION-BASED ASSAYS OF CLONAL IMMUNOGLOBULIN GENE AND TCF3-PBX1 TRANSCRIPTS IN THE DETECTION OF MINIMAL RESIDUAL DISEASE IN T(1;19) ACUTE LYMPHOBLASTIC LEUKEMIA

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Introduction: Quantification of clonal antigen-receptor gene rearrangements by real time polymerase chain reaction (QPCR) is considered to be the standard methodology for minimal residual disease (MRD) monitoring in acute lymphoblastic leukemia (ALL). An alternative less labor-intensive and less expensive approach is the quantification of fusion transcripts in those carrying recurrent chromosome translocations by reversion transcriptase PCR (RQ-PCR) assay. Previous studies showed that the correlation between clonal sensitivity of 10^-4 < 0.01%. Group on MRD detection in ALL. MRD-negative was defined as assay for according to the Europe Against Cancer Program, and QPCR performed for each sample: RQ-PCR with TaqMan after therapy in 13 children and 4 adults with t(1;19) ALL. Totally, samples were collected at diagnosis and at different time points after therapy in 13 children and 4 adults with t(1;19) ALL. Totally, 44 follow-up samples were analyzed. Two PCR-based assays were performed for each sample: RQ-PCR with TaqMan assay using ABL gene as control for measurement of TCF3-PBX1 fusion transcripts according to the Europe Against Cancer Program, and QPCR assay for Ig targets following the guidelines of the European Study Group on MRD detection in ALL. MRD-negative was defined as < 0.01%. Results: Among the 17 patients, 13 had a QPCR assay for Ig targets with a sensitivity of 10^-4 and the remaining 4 had a sensitivity of 10^-3. The detection sensitivity of RQ-PCR for TCF3-PBX1 was >10^-3. After induction treatment, 2 of 3 adult patients were MRD positive compared with 3 of 13 pediatric patients. A comparison between the two PCR-based assays showed that MRD was both positive in 14 samples and both negative in 30 samples (Kappa 1; concordance 100%, Figure 1). In addition, the MRD results of both PCR-based assays were fully concordant in the ranges between 10^-3 and 10^-4, with the differences being less than 1 log in all MRD-positive samples. A lower sensitivity of QPCR assay for Ig targets (10^-4) compared to that for TCF3-PBX1 transcripts (10^-3) was observed in 12 of the 30 MRD-negative samples, suggesting that TCF3-PBX1 transcript assay was more sensitive as compared with Ig QPCR assay. Conclusions: Our study showed that the correlation between clonal Ig QPCR assay and TCF3-PBX1 RQ-PCR assay was well concordant. RQ-PCR assay for TCF3-PBX1 transcripts is more cost-effective in MRD monitoring for patients with t(1;19)/TCF3-PBX1 ALL.

Figure 1. MRD results obtained from quantitative PCR for clonal Ig genes and TCF3-PBX1 fusion transcripts. ‘o’ indicates follow-up samples for children and ‘x’ for adults.
Conclusions: Our results suggest that a significant number of the Qatari pediatric population exhibits mutational changes responsible for the increasing prevalence of α-thalassemia in the population. However, roughly 62% of the children pooled failed to exhibit any mutation, despite a clinical presentation highly suggestive of α-thalassemia. This suggests the possibility of other existing mutations in the Qatari pediatric population that are yet to be elicited.

414 THERAPY-RELATED MYELOID NEOPLASM WITH A T(5;14)(Q33;Q32) AND PDGFRB REARRANGEMENT AFTER TREATMENT FOR ACUTE PROMYEOLOCYTIC LEUKEMIA

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Introduction: The introduction of all trans retinoic acid (ATRA) has been a major breakthrough in the treatment of acute promyelocytic leukemia (APL) with t(5;17)(q22;q11). The combination of ATRA and anthracycline-based chemotherapy results in high rates of complete remission and survival in the patients. However, combined ATRA and chemotherapeutic drugs increase the risk of therapy-related myeloid neoplasms (t-MN), which has an extremely poor clinical outcome. Myeloproliferative neoplasms with translocation involving PDGFRB in 5q33 are rare diseases. Many partner genes of PDGFRB have been described, but ETV6 is the most common. We report the case of a t-MN with a t(5;14)(q33;q32) with PDGFRB rearrangement after treatment for APL.

Methods: Results: A 51-year-old Korean male patient treated for APL who developed t-MN with a t(5;14)(q33;q32) while on maintenance therapy. The patient initially diagnosed with APL in October 2010. Induction treatment with ATRA and induction chemotherapy induced complete hematologic remission and molecular remission. Response to therapy was monitored by fluorescence in situ hybridization (FISH), reverse-transcriptase PCR for PML-RARα rearrangement. In October 2013 on maintenance therapy, however, conventional cytogenetics revealed t(5;14)(q33;q32). FISH analysis demonstrated that PDGFRB was involved in the translocation. No PML-RARα fusion gene was detected in marrow and peripheral blood mononuclear cells. t-MN has an extremely poor clinical outcome. Conclusions: We report the case of a t-MN with a t(5;14)(q33;q32) with PDGFRB rearrangement after treatment APL and this case stresses the importance of performing conventional karyotyping on a regular basis in all treated APL patients for the early detection of chromosomal aberrations indicative of the development of t-MN.

416 OVEREXPRESSIONED LET-7A-3 IS ASSOCIATED WITH POOR OUTCOME IN ACUTE MYELOID LEUKEMIA

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Introduction: Dysregulation of microRNA let-7a-3 has been identified in several solid tumors and is associated with prognosis of patients. However, the pattern of let-7a-3 expression and the impact on prognosis has not yet been studied in acute myeloid leukemia (AML). Methods: The expression status of let-7a-3 was analyzed in AML using real-time quantitative PCR. Results: Overexpression of let-7a-3 was identified in 25 of 102 (25%) de novo AML. There was no significant difference in age, blood parameters, FAB/WHO subtypes, karyotype risks and nine gene mutations (FLT3-ITD, NPM1, C-KIT, IDH1/IDH2, DNMT3A, C/EBPA and N/KRAS) between patients with and without let-7a-3 overexpression (P > 0.05). The patients with let-7a-3 overexpression had similar rates of complete remission (CR) as those without let-7a-3 overexpression (50% versus 56%, P = 0.693). Although the overall survival (OS) of AML patients with let-7a-3 overexpression (median 12 months) was shorter than those without overexpression (median 25 months), the difference was not statistically significant (P = 0.228). However, among those 51 obtained CR, patients with let-7a-3 overexpression had significantly shorter OS than those without let-7a-3 overexpression (P = 0.029). The difference in relapse-free survival (RFS) was also significant between two groups (P = 0.005). Conclusions: let-7a-3 overexpression is a common event and is associated with poor clinical outcome in AML.

418 SOMATIC MOSAICISM CAUSING PK DEFICIENCY IDENTIFIED BY NEXT GENERATION SEQUENCING: IMPLICATIONS FOR GENETIC COUNSELING

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Introduction: Chronic hemolytic anemia due to pyruvate kinase (PK) deficiency (MIM 609712) is a rare autosomal recessive disorder caused by mutations in the PKLR gene. From a clinical point of view, PK-deficient patients manifest a highly variable phenotype, ranging from severe neonatal jaundice and anemia at birth with severe transfusion dependent chronic hemolysis, to moderate hemolysis with exacerbation during infection, or fully compensated hemolysis without apparent anemia. An estimated 3% of PK-deficient patients present with a de novo mutation. We report for the first time a case of PK deficiency associated to vertical transmission of a de novo somatic mutation in PKLR. Methods: RBC enzymes activities for PK and HK were measured according to ICSH. To relate the measured PK activity to the mean age of the red blood cells the PK/HK ratio was also calculated. Other causes of chronic hemolysis (i.e. defects on red blood cell membrane and haemoglobin) were ruled out. DNA analysis of PKLR was performed by conventional Sanger sequencing. Massive parallel sequencing of PKLR was performed on DNA from peripheral blood, mucosal swabs, 24 hours urine, and sperm samples from selected family members. Results: The proband was an 18 month old boy suffering from severe transfusion-dependent chronic hemolytic anemia due to PK deficiency. PK activity in the mother resembled...
the PK-deficient carrier state whereas in the father was normal. DNA sequence analysis revealed the proband to be compound heterozygous for two missense mutations in PKLR: c.359C>T (p.Ser120Phe) and c.1168G>A (p.Asp390Asn). The c.359C>T change was found to be inherited from the mother. However, the c.1168G>A mutation could not be detected in blood father’s DNA, despite confirmed father-mother-child allelic inheritance. Subsequent massive parallel sequencing of the region encompassing nt c.1168 on DNA from different tissues of the father indicated that this mutation has arisen postzygotically, thereby producing parental mosaicism. Conclusions: The methodology used allowed the detection of a somatic mutation in the father that was vertically transmitted to his son. This enabled us to unravel an exceptional case of a rare autosomal disease and, thereby, enabled proper genetic counseling. Accordingly, a somatic mutation screening should be performed in all cases where an apparent de novo mutation is identified during the genetic diagnosis of PK-deficiency. This can be applied to other genetic diseases, either recessively or dominantly inherited, as some cases of vertical transmission of somatic mutations from the parents have been described, at least for dominant inherited diseases.

420 MOLECULAR DETECTION OF MOST INTERNATIONALLY REPORTED MUTATIONS IN EGYPTIAN FANCONI ANEMIA PATIENTS
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Introduction: FA-A is the most frequent complementation group representing approximately two-thirds of the patients in the majority of countries. Aim of the work: screening for the most internationally reported mutations of FANCA gene in the Egyptian Fanconi anemia patients and evaluate a correlation between clinical and cytogenetic results. Methods: Thirty Egyptian FA (fanconi anemia) cases, descending from unrelated consanguineous pedigrees were recruited from the Hereditary blood disorders(HBD) clinic, NRC. All patients had positive chromosomal breakage studies with diepoxybutane (DEB) confirming the diagnosis of FA. Amplification of FANCA gene exons of interest by PCR, Multiplex PCR, Restriction Analysis, DNA sequencing for patients and controls was done. Results: 20 % (6/30) of our patients had homoyzgous deletion of (12-31) exons of the FANCA gene, these patients presented with severe phenotype, 67% were born with low birth weight. All patients had cafe` au lait spots, hyper pigmentation, and skeletal abnormalities in 83%. Moreover, chromosomal breakage (DEB test) in this group was with an average of 11.5 break/ cell. No 2574 C G (858R) mutation in exon 27 or c.3788_3790 del TCT mutation in exon 38 in the FANCA gene was detected in our studied patients. Moreover, no new mutations were detected in exons 34 and 43 of the FANCA gene. Conclusions: This is the first step in defining the mutation spectrum of FA in Egyptian patients. 20 % (6/30) of our patients had homoyzgous deletion of exons (12-31) of the FANCA gene, these patients presented with severe phenotype, Further studies on a bigger number of patients are highly recommended to be able to detect all the mutations affected in the Egyptian population.

422 BCR-ABL FUSION TRANSCRIPTS’ FREQUENCIES IN SUDANESE CML PATIENTS
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Introduction: Variable BCR-ABL fusion transcripts associated with chronic myeloid leukemia (CML) has been reported in different ethnic groups, but not among Sudanese neither African population. Methods: This study was designed to determine the co-expression frequencies of the p210 and p190 in Sudanese CML patients. A total of 112 CML Sudanese patients 65 Male (58%), and 47 Female (42%); age ranged between 2 and 70 year-old (median 38) were enrolled in the present study. We performed nested RT-PCR followed by transcript analysis using QRT-PCR on patient’s venous blood and bone marrow samples. Results: Nested RT-PCR was positive in 112 cases while QRT-PCR was only successful in 109 cases. Out of the 109 cases 35(38.4%) expressed one or both P210 BCR/ABL rearrangements (b2a2 and b3a2). Of the 35 patients, 21(60%) patients expressed b2a2 only, 6(17.1%) expressed b3a2 only, and 8(22.8%) expressed both transcripts b2a2/b3a2. The remaining 74 patients revealed a transcript combination of P190 BCR/ABL and P210 BCR/ABL (e1a2/b2a2/b3a2), of which 19(25.7%) patients had all the transcripts (e1a2/b2a2/b3a2), 33(44.6%) revealed two transcripts (e1a2/b2a2), and the remaining 22(29.7%) patients had (e1a2/b3a2). Conclusions: Our results showed differences in the frequencies of BCR-ABL fusion transcripts compared with previously reported studies. This may reflect the sensitivity of the detection techniques used. The possibility of genetic differences between the populations involved in this study,or the alternative splicing that may lead to an increased co-expression resulting in phenotypic variation with clinical courses different from classical CML.

424 A COMPARISON OF HIGH RESOLUTION MELTING, ALLELE-SPECIFIC PRIMING AND SANGER SEQUENCING FOR THE DETECTION OF BRAFV600E MUTATION IN HAIRY CELL LEUKAEMIA FROM DIFFERENT HAEMATOLOGICAL SPECIMENS
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Introduction: Recently, the BRAFV600E mutation was discovered as a highly specific and sensitive marker for hairy cell leukaemia (HCL) and a potential target for drug therapy. However, the quality of clinical specimens is often suboptimal for genotypic analysis due to a small number of hairy cells in blood and bone marrow aspirate. This study aims to assess the usefulness of three molecular techniques for the detection of theBRAFV600E mutation in HCL from different types of haematological samples. Methods: We assessed the performance of high resolution melting (HRM), allele-specific priming (ASP) and Sanger sequencing (SS) for BRAFV600E detection in 17 unenriched HCL samples: blood (n=7), marrow aspirate (n=3), ethylenediaminetetraacetic acid (EDTA)-decalcified trephine biopsy (n=2), formic acid (FA)- decalcified trephine biopsy (n=5).
Results: In peripheral blood and marrow aspirate samples where DNA was well preserved, all three molecular techniques showed analysable results in all 10 HCL except for one sample in which HRM result was not analysable. In formalin-fixed, paraffin-embedded and decalcified trephine biopsies, DNA preservation was less optimal. High resolution melting analysis failed to produce an analysable result in any of the seven trephine biopsies tested. The poor performance of HRM on trephine biopsies was not related to the method of decalcification, the length of storage or the extent of involvement. Allele-specific priming and SS demonstrated a better analytical ability for the mutation. The results showed that for blood and marrow aspirate, both HRM and ASP had a very high analytical sensitivity (1% tumour load), diagnostic sensitivity (100%) and specificity (100%) in analysable samples. Sanger sequencing had a lower analytical sensitivity (4% tumour load), resulting in false-negative analysis in some cases. High resolution melting was technically the simplest and had the shortest turn-around-time of 2 hours. In decalcified trephine biopsies, HRM was not useful, while SS was least demanding on sample DNA quality for a successful analysis. However, none of the three techniques showed satisfactory diagnostic performance for trephine biopsies. Conclusions: High resolution melting is a cost-effective technique for initial screening of the BRAFV600E mutation in blood and marrow aspirate samples of suspected HCL patients. Atypical cases can be confirmed by ASP or SS. A robust detection method for this mutation on decalcified trephine biopsies still awaits development and validation.

426 QUANTIFICATION OF BCR-ABL1 TRANSCRIPTS ON BONE MARROW SAMPLES USING THE GENEXPERT BASED V1 CARTRIDGE SYSTEM

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Introduction: The European LeukemiaNet 2013 guidelines recommend monitoring of BCR-ABL1 in CML patients on tyrosine kinase inhibitors therapy at distinct time points by quantitative PCR. The GeneXpert system (Cepheid) using V1 cartridges allows quantification of e13-/e14a2 transcripts on the International scale (IS) on peripheral blood (PB). Analysis of bone marrow (BM) samples is however not supported by the manufacturer. The aim of this study was to quantify BCR-ABL1 transcripts in BM using GeneXpert. Methods: Firstly, a comparative study between GeneXpert and TaqMan-RQ-PCR was done on 37 BM samples. GeneXpert analysis were performed according manufacturer recommendations, however using only 20 µL of sample, based on previously published data (Lopez-Jorge CE. et al, 2012). All results were expressed as BCR-ABL1 %IS. To compare results a categorical interpretation with following logarithmic intervals was used: 10-1%, 1-0.1%, 0.1-0.01%, 0.01-0.001%, <0.001% and “not detected”. Secondly, 8 additional BM samples that tested negative on GeneXpert by using 20 µL were rerun on GeneXpert with 200 µL (the prescribed PB volume). Results: Comparison of GeneXpert and TaqMan data shows that 19/37 samples (51.4%) had the same categorical interpretation and a difference of one was found in 14 samples (37.8%). Ten out of these 14 samples had a MMR (major molecular response) by both methods, 3/14 showed no MMR by both methods and 1/14 was discordant; no MMR with TaqMan versus MMR with GeneXpert. Four samples had a ≥ two categorical difference; three had MR4 (molecular response of ≥4 log reduction) with TaqMan but no BCR-ABL1 with GeneXpert, and one sample had no MMR with TaqMan (0.221%IS) while GeneXpert showed no detectable BCR-ABL1 (due to suboptimal PCR, as indicated by high ABL Ct). Double positive samples (24/25) showed comparable stratification of molecular response (no MMR versus MMR). Comparing 20 µL versus 200 µL of BM sample for GeneXpert analysis: 2/8 were negative with 20 µL but low positive with 200 µL, while in 6/8 samples “BCR-ABL1 was not detected”. The limit of detection in these latest samples was always lower when using a higher sample volume (p-value = 0.007). Conclusions: GeneXpert quantification of BCR-ABL1 transcripts in BM shows comparable stratifications of molecular responses in case of positive results. However, when using only 20 µL of BM, the number of complete molecular remissions is overestimated: therefore, 200 µL (similar to PB) might be recommended for analysis of BCR-ABL1 transcripts in BM on a GeneXpert system.

428 SF3B1 MUTATION IS A RARE EVENT IN CHINESE PATIENTS WITH ACUTE AND CHRONIC MYELOID LEUKEMIA

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Introduction: Somatic mutations of SF3B1 gene have recently been identified in myelodysplastic syndrome and chronic lymphocytic leukemia. The frequency and clinical relevance of SF3B1 mutations have been rarely studied in acute myeloid leukemia (AML) and chronic myeloid leukemia (CML). The present study was aimed to analyze the frequency of SF3B1 mutations in AML and CML. Methods: High-resolution melting analysis (HRMA) was established to detect the mutation hotspots (codon E622, H662, K666, and K700) of SF3B1 gene in 275 AML and 81 CML patients. Results: Heterozygous SF3B1 mutations were detected in three AML patients by HRMA. Direct DNA sequencing identified one K666T, one K666N and one K700E mutation. All three AML patients had normal karyotypes. One case also had NPM1 and DNMT3A mutations, one had FLT3 internal tandem duplication and DNMT3A mutations, and the other had NPM1 mutation. No SF3B1 mutations were detected in CML patients. Conclusions: SF3B1 mutation is a rare molecular event in Chinese AML and CML patients.

500 A NEW STRATEGY FOR AUTOMATIC IDENTIFICATION OF ATYPICAL LYMPHOID CELLS FROM PERIPHERAL BLOOD CELLS IMAGES

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Introduction: Morphological analysis and classification of peripheral blood (PB) images to support medical diagnosis is being the subject of active research. Atypical lymphoid cells are the most difficult and challenging pathological cells to classify using morphology features only. The main objective of this work was to design a new methodology in order to achieve a robust and accurate automatic classification of atypical lymphoid cells using PB cell digital images. Methods: We analyzed 1834 digital images
of individual lymphoid cells from PB films stained with May-Grünewald-Giemsa and obtained in the CellaVision DM96. Among them, 180 images belong to healthy patients (N), 301 to patients with Hairy Cell Leukemia (HCL), 542 to patients with Chronic Lymphocytic Leukemia (CLL), 401 to patients with Mantle Cell Lymphoma (MCL), 334 to Follicular lymphoma (FL) and 75 were B-cell Prolymphocytes (BPL). We implemented clustering of color components and Watershed Transformation to segment the nucleus, the cytoplasm and the peripheral cell region. We extracted 1429 features from these regions: 1352 color and texture features from CMYK color space, 76 geometric features and 1 from the external profile of the cytoplasm. Afterwards, we applied information theory feature selection in order to choose the best 18 features. These were used by the supervised learning algorithm Support Vector Machine (SVM) with a radial basis function kernel to classify the different subtypes of lymphocytes. Results: We performed a 10 fold cross-validation of SVM. This technique randomly divides the data set into 10 equal size subsets. A single subset is used as the validation date, while the remaining data are used for training. Then, the process is repeated 10 times, with each subset as the validation data. The accuracy of this procedure was 97.98%. Figure 1 shows the sensitivity and specificity for each lymphoid subtype in the entire validation process. Conclusions: Our strategy includes a robust segmentation method, a complete feature extraction and a successful classification procedure. It is important to remark the high number (6) of groups involved in the classification. The use of this methodology may be a significant support tool for initial lymphoid neoplasms diagnosis from peripheral blood morphologic analysis.

Methods: Haematology analysers, measure mean platelet volume (MPV), to determine platelet size. Additionally the ADVIA2120 (Siemens) analyzer has increased the range of platelet data that can be derived during the full blood count. In addition to platelet count and MPV, this analyzer has added platelet-crit (PCT), platelet distribution width (PDW), mean platelet component concentration (MPC), platelet component distribution width (PCDW), mean platelet mass (MPM) and platelet mass distribution width (PMDW). This has been possible because of the two-dimensional approach used by the ADVIA 2120 employing flow cytometry to analyze low and high angle scatter of light by platelets. As a consequence of this technology large platelets up to 60fL will be included in the count whilst the measurement of refractive index eliminates contamination from small red cells or fragments. Results: The morphological aspects of Inherited Thrombocytopenia are characteristic and can help differentiate different disorders. They range from the small platelets found in Wiskott-Aldrich Syndrome to the giant size platelets of Bernard-Soulier Syndrome. Conclusions: This poster shows many examples of Platelet and Megakaryocyte Morphology as well as analyser results, and may provide a diagnostic aid for Inherited Thrombocytopenias.

504 ENUMERATION OF SCHISTOCYTES USING THE DM-96 AUTOMATED MICROSCOPE
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Introduction: The schistocyte working group of the ICSH recommends microscopic evaluation of the peripheral blood smear for schistocyte enumeration, despite introduction of automated schistocyte counting. Precise morphological criteria for schistocytes enumeration have been provided by the ICSH. However, despite the standardization efforts, inter- and intra-observer bias remains a critical issue. The selected region on the blood smear influences the identification of schistocytes. Traditionally, schistocyte enumeration is performed using a microscope estimated as a percentage of 1000 RBCs. The latter is prone to inter and intra-reader variability, as often an estimation of the RBCs is made. We have explored the automated microscope (DM-96) for schistocyte enumeration and its value for standardization. Methods: We compared schistocyte enumeration between manual microscopy and automated microscopy in 30 patient samples. The identification of the schistocytes was based on the ICSH definition. The manual schistocyte count was performed using a medium magnification (50x10x). For the automated microscopy, the DM-96 was used. Schistocytes were counted in the platelet evaluation module of the DM-96. We have used this module to estimate the amount of schistocytes per µl. In combination with the RBC count per µl, the amount of schistocytes in each sample was calculated per 1000 RBC. RBCs were counted using the XN-3000 analyzer. To evaluate the inter-observer agreement using the automated microscope, an internal quality round was organized. Twelve laboratory professionals analyzed 3 samples and enumerated the schistocytes using the DM-96. To evaluate intra-observer agreement using the automated microscope, the standard deviation (SD) of duplicate measurements for one observer was calculated (n=2x30). Results: A good correlation between the traditional microscope and the automated microscope (r=0.97) was found. The mean schistocyte count using manual microscopy is not significantly different from the automated microscope (mean difference = 0.8). The difference between both methods was not significant (p>0.05). The inter-
individual coefficient of variation for each sample, respectively 10.5, 8.9 and 15.0 %. The intra-observer variation equals 25.8 %. As the mean number of schistocytes per sample was 4 (range: 1–20; n=30), the mean SD was 1 schistocyte (not clinically relevant). Intra-observer variation using the manual microscope was 38.9 %. **Conclusions:** Our study has shown that the inter-and intra-individual variation of the microscopic schistocyte count is decreased using automated microscopy. Counting schistocytes on the automated microscope is a good alternative for the traditional microscope and leads, when the observers are properly trained, to significant decrease in inter-and intra-observer variation in schistocyte count.

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**506 CONTRIBUTION OF THE PERIPHERAL BLOOD (PB) CELL IMAGES OBTAINED IN THE CELLAVISION DM96 TO THE INFECTIOUS DISEASES DETECTION**

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**Introduction:** Mortality and morbidity in infectious diseases can be reduced with early diagnosis. Automated systems for leukocyte recognition based in artificial neural network techniques such us the CellaVision DM96 are currently available and are used in clinical laboratories. The objective of this work was to evaluate if the digital blood cell images obtained in the Cellavision DM96 may contribute to the quick diagnosis in some infectious diseases. **Methods:** During 2013 we analysed 190 samples from patients with suspected Malaria and 320 from patients with other infectious diseases. Blood samples were collected into tubes containing K3EDTA as anticoagulant and analyzed by a cell counter Sysmex XN.PB films were stained automatically with May-Grunwald-Giemsa. The slides were loaded into the CellaVision DM96 analyzer. PB films were stained automatically with May Grünwald-Giemsa. The slides were loaded into the Cellavision DM96 obtaining digital images of the blood cells at high magnification. **Results:** From a total of 44 PB slides in which we found such inclusions in the neutrophils, we found that 27 slides were from patients with positive CMV antigenemia (61%). In this group 6/27 were also HIV positives. All the 27 CMV patients received antiviral therapy with drugs that act on the genome replication. In addition, one blood film was from an HIV-positive and CMV-negative patient who did not receive any treatment. In one patient CMV and HIV positive we detected the inclusions also in the monocytes. Taking into account the remaining 16 PB films, 12 were from patients with different neoplasms: a) hematologic: myelodysplastic syndrome (2), acute myeloid leukemia (1), myeloproliferative neoplasms (2), lymphoid neoplasms (6), b) non hematologic: breast cancer (1). One slide was from a patient with marrow aplasia. The remaining 3 slides were from blood patients after kidney (2) or hepatic (1) transplant. All of the patients included in this group received chemotherapy, immunosuppressive therapy or both. **Conclusions:** Development of Howell-Jolly body-like inclusions in blood neutrophils are not infrequently observed in patients receiving antiviral drugs that act on the genome replication, patients on anticancer chemotherapeutic agents or HIV-positive individuals in the absence of any chemotherapy drug.

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**508 HOWELL-JOLLY BODY-LIKE INCLUSIONS IN PERIPHERAL BLOOD NEUTROPHIL IMAGES IN IMMUNOSUPPRESSED PATIENTS**

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**Introduction:** Neutrophils that have detached nuclear fragments equivalent to the Howell-Jolly bodies of erythrocytes have been described in association with azathioprine, chemotherapy or ganciclovir therapy. These inclusions showed positivity for the Feulgen staining. Our aim was to analyze the characteristics of the patients that developed nuclear fragment inclusions in blood neutrophils detected in our hematology laboratory during one month period. **Methods:** During November 2013 we detected a total of 44 peripheral blood (PB) films in which we observed Howell-Jolly body like inclusions in the neutrophils. Blood samples were collected into tubes containing K3EDTA as anticoagulant and blood cell counts were done using the Advia 2120 (Siemens) analyzer. PB films were stained automatically with May Grünwald-Giemsa. The slides were loaded into the Cellavision DM96 obtaining digital images of the blood cells at high magnification. **Results:** From a total of 44 PB slides in which we found such inclusions in the neutrophils, we found that 27 slides were from patients with positive CMV antigenemia (61%). In this group 6/27 were also HIV positives. All the 27 CMV patients received antiviral therapy with drugs that act on the genome replication. In addition, one blood film was from an HIV-positive and CMV-negative patient who did not receive any treatment. In one patient CMV and HIV positive we detected the inclusions also in the monocytes. Taking into account the remaining 16 PB films, 12 were from patients with different neoplasms: a) hematologic: myelodysplastic syndrome (2), acute myeloid leukemia (1), myeloproliferative neoplasms (2), lymphoid neoplasms (6), b) non hematologic: breast cancer (1). One slide was from a patient with marrow aplasia. The remaining 3 slides were from blood patients after kidney (2) or hepatic (1) transplant. All of the patients included in this group received chemotherapy, immunosuppressive therapy or both. **Conclusions:** Development of Howell-Jolly body-like inclusions in blood neutrophils are not infrequently observed in patients receiving antiviral drugs that act on the genome replication, patients on anticancer chemotherapeutic agents or HIV-positive individuals in the absence of any chemotherapy drug.
510 LINKING TESTING-TO-CLINICAL GOALS - A RISK STRATIFIED PROCESS REDESIGN SCHEME TOWARDS IMPROVING MANUAL BLOOD FILM REVIEW (MBFR) SERVICES IN A LARGE TERTIARY HOSPITAL LABORATORY

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Introduction: A hematopathologist’s review and comment (HPCOM) on blood film can be of critical importance in the initial diagnostic work up of a hematological disease. Recent feedback from the clinical users verified that HPCOM information is generally not available for timely clinical decision making and patient management. This paper presents the development and implementation of a process redesign project, aiming to improve the turn-around-time (TAT) of critical HPCOM results to meet clinical user’s expectation in a high volume large tertiary hospital laboratory. Methods: The key components of the logic model include: (1) A risk stratification scheme to separate blood film morphology abnormalities - (i) minor/low diagnostic specificity entities, as Disease Associated Abnormalities (DAA), or (ii) major/clinical significant entities, as Disease Defining Abnormalities (DDA). For DAA, the use of an intelligent data manager (Middleware™), permits the development of customized alert codes/decision functions for some numerical abnormalities, as a diagnostic aid, based on pre-defined logics in the transactions of laboratory information to clinical users. (2) For DDA, the implementation goal is to upkeek/advance the diagnostic acuity of the front-line technologists. An Inter-professional Learning (IPL) model is designed to foster a codependent learning opportunity between the technologists and pathologists. The combined strategies aim to cope with the technologists’ workload in managing DAA cases, while enhancing their core competence in recognizing critical morphological abnormalities and refer them to a pathologist for review on a priority basis. Anonymous LIS data at Vancouver General Hospital before and after program implementation were extracted and analyzed for performance evaluation. Results: Despite an increase in CBC test volume of 6.5% (23,843 vs. 25,380 respectively) in the study period (January - 2013 and 2014), before and after program implementation, the rate of MBFR by technologists remained constant (4,337 vs. 4,666; 18.2% vs. 18.4% respectively). The HPCOM cases declined substantially (1,024 vs. 839; 4.3% vs. 3.3% of total respectively). A significant reduction in TAT for HPCOM was evident: (1) Median TAT (14.6 vs. 6.8 hours respectively; p<0.0001), (2) TAT cut-off ≤6 hours (27.3% vs. 46.1% respectively; p<0.0001. Conclusions: The standard MBFR process relies on a sequential transactional pathway, resulting in significant time delay in the final release of HPCOM information to clinical users. This is a proof-of-concept model which demonstrated that risk stratification/prioritization and knowledge sharing in a case-based learning environment are effective strategies and can improve the clinical goals of MBFR services.

512 DIGITAL MORPHOLOGY: A PROVEN CONCEPT

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Introduction: The analysis of blood morphology is of great diagnostic importance to the clinician. An exciting development in this field is the introduction of digital microscopy systems, enabling the automated morphological assessment of blood smears. This includes an automated peripheral blood cell differential and blast cell detection of all nucleated blood cells. Methods: A large database containing over 1.5 million leukocytes was set up and analyzed by the digital microscope DM96 to determine the accuracy of its pre-classification performance compared to manual classification (the gold standard) and its potential for auto-validation. Results: Regression coefficients ranging from 0.95 to 0.99 were found for neutrophils, lymphocytes and eosinophils and coefficients between 0.87 and 0.88 for monocytes and basophils. Blast detection showed a sensitivity of 100%. Conclusions: The pre-classification performance of the DM96 was shown to be equal to the manual classification of the main blood cell classes in peripheral blood smears. In addition, auto-validation of blood smears is possible with the use of confidence limits.

514 INTER-LABORATORY REPRODUCIBILITY OF DIGITAL BLOOD MORPHOLOGY

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Introduction: Differential counting of peripheral blood cells is an important diagnostic tool. However, manual morphological analysis using the microscope is time-consuming and requires highly trained personnel. The digital microscope (DM) is capable of performing an automated peripheral blood cell differential, which is as reliable as manual classification by experienced laboratory technicians in classifying the five main peripheral blood cell categories and recognition of blast cells. To date, information concerning the inter-laboratory variation and quality of cell classification by independently operated digital microscopy systems is limited. Methods: We set out to compare four independently operated digital microscope systems, stationed at four different medical centres. We compared their ability to classify the five main peripheral blood cell classes (segmented neutrophils, basophils, lymphocytes, eosinophils and monocytes) and detection of blast cells in 200 samples. Results: The percentage of detected segmented neutrophils, eosinophils, basophils, lymphocytes, monocytes and blast cells ranged from 0 to 94%, 0 to 28%, 0 to 6%, 0 to 92.5%, 0 to 37.5% and 0 to 83.5% respectively. Set against the averaged results of all four systems, the segmented neutrophils showed R²-values ranging between 0.97 and 0.98, the eosinophils between 0.82 and 0.88, the lymphocytes between 0.96 and 0.98, the monocytes between 0.89 and 0.91 and the blast cells between 0.99 and 1.00. The R²-values for the basophils were between 0.49 and 0.59. Conclusions: This study shows that independently operated digital microscopy systems yield reproducible pre-classification results when determining the percentages of segmented neutrophils, eosinophils, lymphocytes, monocytes and blast cells in a peripheral blood smear. Detection of basophils was hampered by the low incidence of this cell class in the samples.

600 IMMEDIATE FIXATION OF TREATED BLOOD SAMPLES ENABLES FLOW CYTOMETRIC MEASUREMENT OF PLATELET AGGREGATION AND PLATELET-LEUCOCYTE CONJUGATE FORMATION IN SMALL VOLUMES OF BLOOD.

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Introduction: The current gold standard in platelet function testing, light transmission aggregometry (LTA), is time- and labour-intensive, and uses platelet-rich plasma (PRP), which requires a significant volume of blood sample and is not suitable in patients with low platelet counts. Alternative methods exist to assess platelet function in whole blood, however, blood samples must be processed within a limited time after venepuncture using specialist equipment. We have developed a method to assess platelet aggregation and platelet-leucocyte conjugate formation in fixed whole blood samples, which can be be be can be stored prior to analyses. Here we evaluated this assay performed in the 96-well plate format which offers the advantage of using a reduced blood volume. Methods: Platelet function was assessed in whole blood obtained from healthy volunteers, using 96-well plates coated with 4µl of the following agonists: arachidonic acid (AA, 0.03-1mM), ADP (0.3-30µM), collagen (0.1-10µg/ml) and TRAP (0.1-10µM), or vehicle. To see if the assay can detect decreased platelet responses whole blood was also incubated with aspirin, cangrelor or their combination. 46µl of whole blood was added to each well and the plate was shaken for 5min at 1000 rpm at 37°C; a fixative solution AGGFix (Platelet Solutions Ltd., Nottingham, UK) was applied to stop platelet aggregation and stabilise samples for up to 9 days prior to analyses; aggregation was assessed by flow cytometry as a decrease in the number of single platelets. Platelet-leucocyte conjugates analysis in the same whole blood samples was completed within 3 days after fixation. Results: In 10 healthy volunteers aggregation assessed in duplicate was robust and reproducible (CV<10%). As expected, cangrelor induced a profound inhibition of ADP-induced aggregation and aspirin dose-dependently inhibited platelet responses to AA. However, at the highest AA concentration (1mM) considerable aggregation was observed, which was abolished by addition of cangrelor, suggesting that ADP, possibly appearing from red cells, is responsible for the aggregation response. Collagen- and TRAP-induced aggregation was also impaired to a different extent by in vitro treatment with either antiplatelet agent. Platelet-leucocyte conjugate formation was readily measured in the same whole blood samples and was reduced by platelet inhibition. Conclusions: Platelet aggregation and conjugate formation assessed by flow cytometry in fixed whole blood might be useful for investigation of platelet function, especially in individuals where blood sample volume is limited. Further investigation to determine the clinical utility of the assay in detecting acquired and inherited defects in platelet function is warranted.

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ABT-737 INDUCES A DISTINCT PHENOTYPE OF APOPTOTIC PLATELETS LACKING PRO-INFLAMMATORY POTENTIALS
Mehran Ghasemzadeh1, Elteteramolsadat Hosseini1, Shaun Jackson2
1Blood Transfusion Research Center, High Institute for Education and Research in Transfusion Medicine, Tehran, Iran. 2Australian Centre for Blood Diseases, Monash University, Melbourne, Australia

Introduction: Further activation of platelets during thrombus development is associated with a sustain increase in internal calcium leading to externalization of negatively charged phospholipids on platelet surface and eventually platelet apoptosis. Although formation of Ps-positive platelets is associated with the decrease in their adhesive and pro-inflammatory functions, whether these malfunctions are related to post-activation changes or also can be induced by direct effect of apoptosis is still ill-defined. To address this question, applying ABT-737, a BH3 mimic, induces apoptosis without cell activation, we investigate the sole effect of apoptosis on pro-inflammatory potentials of platelets. Methods: Agonist-induced P-selectin expression, Ps exposure and PAF generation on platelets surface. Resting or stimulated platelets with ADP, TRAP, CRP/Thimerosal or ABT-737 in the presence of either labelled P-selectin antibody or annexin V were monitored by flow cytometry analysis. The level of PAF was also quantitated by mass spectroscopy for each platelets samples. Platelet-dependent induction of neutrophil Mac-1 expression. Before-mentioned panels of platelets were incubated with neutrophils at increasing platelet/neutrophil ratios in the presence of anti-human CD11b to monitor Mac-1 expression by flow cytometry. Time-dependent induction of neutrophil Mac-1 expression by platelets. Platelets were also incubated with neutrophils at a platelet/neutrophil ratio of 10/1 for the indicated time course, in the presence of anti-Human CD11b to monitor Mac-1 expression. Results: P-selectin expression, Ps exposure and PAF generation in fully TRAP-activated platelets were unique as they showed the highest Ps level, in the absence of significant P-selectin expression or PAF generation. At all platelet/neutrophil ratios used, only P-selectin positive platelets (those generated after treatment with TRAP and CRP/Thimerosal) induced a significant increase in Mac-1 expression. CRP/Thimerosal activated platelets are more effective than TRAP activated one while their P-selectin expression is the same. Neither ADP nor ABT-737 treated platelets had any significant impact on the level of Mac-1 expression even after longer incubation time. Conclusions: Platelet apoptosis without proceeding of activation steps cannot induce neutrophil activation that suggests no significant pro-inflammatory effects for this phenotype of platelets. The highest levels of PAF generation in fully activated platelets may support the increase in pro-inflammatory potentials of platelets.

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INHIBITORY EFFECTS OF FISH OIL ON PLATELET-ASSOCIATED THROMBIN GENERATION AS MEASURED BY THE CALIBRATED AUTOMATED THROMBOGRAM: AN IN VITRO STUDY.
Jack Harbert, Kelly Pagano, Denae Heartfield, Michael Ero
Machaon Diagnostics, Oakland, CA, USA

Introduction: The effects of omega-3 fatty acids in fish oils have been extensively studied and proven to have measureable cardiovascular benefits, the mechanism of action remains unclear and highly disputed. Platelet functional inhibition is a common target for pharmaceutical agents with a goal of providing protection from thrombotic events, possibly by lowering thrombin generation. This study examined the effects of fish oil on platelet-associated thrombin generation when measured by the endogenous thrombin potential (ETP) and peak height levels using platelet-rich plasma (PRP) on the calibrated automated thrombogram. Methods: Whole blood from six normal volunteers was drawn by syringe and added into 3.2% sodium citrate. Citrated whole blood was centrifuged at 150 g after which PRP was harvested and added into 3.2% sodium citrate. Citrated whole blood was also incubated with aspirin, (0.1-10µM), or vehicle. To see if the assay can detect decreased platelet responses whole blood was also incubated with aspirin, cangrelor or their combination. 46µl of whole blood was added to each well and the plate was shaken for 5min at 1000 rpm at 37°C; a fixative solution AGGFix (Platelet Solutions Ltd., Nottingham, UK) was applied to stop platelet aggregation and stabilise samples for up to 9 days prior to analyses; aggregation was assessed by flow cytometry as a decrease in the number of single platelets. Platelet-leucocyte conjugates analysis in the same whole blood samples was completed within 3 days after fixation. Results: In 10 healthy volunteers aggregation assessed in duplicate was robust and reproducible (CV<10%). As expected, cangrelor induced a profound inhibition of ADP-induced aggregation and aspirin dose-dependently inhibited platelet responses to AA. However, at the highest AA concentration (1mM) considerable aggregation was observed, which was abolished by addition of cangrelor, suggesting that ADP, possibly appearing from red cells, is responsible for the aggregation response. Collagen- and TRAP-induced aggregation was also impaired to a different extent by in vitro treatment with either antiplatelet agent. Platelet-leucocyte conjugate formation was readily measured in the same whole blood samples and was reduced by platelet inhibition. Conclusions: Platelet aggregation and conjugate formation assessed by flow cytometry in fixed whole blood might be useful for investigation of platelet function, especially in individuals where blood sample volume is limited. Further investigation to determine the clinical utility of the assay in detecting acquired and inherited defects in platelet function is warranted.

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(15%EPA/10%DHA), corn oil or 1% bovine serum albumin in tyrode’s buffer (BSA) was added to PRP to achieve a final volume/volume concentration of 1%, 10% or 20%. These solutions were incubated at 37°C for 2 hours. Each sample was then tested using the calibrated automated thrombogram as previously described by Hemker (Thromb Haemost, 2003). Endogenous thrombin potential and peak height were recorded after 60 minutes. Corn oil was selected as a control to mimic the lipid properties of fish oil within the test reaction, and 1% BSA in tyrode’s buffer was selected as an inert volume control. Results: The average ETPs of our experiment are shown in Table 1. There was a significant difference between the BSA group and fish oil group at 20% concentration. There was also a significant difference between the corn oil group and fish oil group. Differences in peak height were not significant (data not shown). Conclusions: The changes seen in both the fish oil and corn oil groups demonstrate a platelet inhibitory effect of fatty acids. The additional reduction in platelet-associated thrombin generation in the fish oil group suggests that the omega-3 fatty acids EPA and DHA have a greater effect than the fatty acids in corn oil. The differences in the fish oil group are dose-dependent. In all, this research suggests that the cardiovascular benefits associated with dietary fish oil supplementation are in part platelet dependent. In vivo research on fish oils in platelet-associated thrombin generation is warranted to further characterize these inhibitory effects.

### Table 1

<table>
<thead>
<tr>
<th>Concentration (%)</th>
<th>1% BSA</th>
<th>Corn Oil</th>
<th>Fish Oil</th>
</tr>
</thead>
<tbody>
<tr>
<td>1%</td>
<td>2024 ± 278</td>
<td>2051 ± 254</td>
<td>1874 ± 285</td>
</tr>
<tr>
<td>10%</td>
<td>3096 ± 139</td>
<td>2090 ± 203</td>
<td>1823 ± 395</td>
</tr>
<tr>
<td>20%</td>
<td>2414 ± 255</td>
<td>2097 ± 132</td>
<td>1728 ± 355</td>
</tr>
</tbody>
</table>

606 RESULTS FROM A STANDARDIZED METHOD FOR COUNTING RETICULATED PLATELETS IN ‘APPEARENTLY DISEASE-FREE’ SUBJECTS FROM 3 INTERNATIONAL SITES

Nigel LLEWELLYN-SMITH1, Benjamin HEDLEY2, Stephen LANG3, Neil MacNAMARA2, David ROSENFELD2, Michael KEENEY2

1Abbott Diagnostics Santa Clara, CA, USA, 2London Health Sciences Centre London, ON, Canada, 3Liverpool Hospital Liverpool, Australia

Introduction: Despite potential as a sensitive marker of thrombopoiesis, the lack of a reference method and standardization materials for Reticulated Platelets (RPs) have hampered adoption. Here we combined two methods (flow platelet enumeration and Thiazole Orange (TO) staining of RNA) to develop a standardized method (previously described at ISLH 2013) to assess typical values in ‘apparently disease-free’ subjects at 3 international sites. Our goal was to test whether 3 different laboratories, using the same protocol on different flow cytometers, could recover statistically comparable results. Methods: The 3 sites were: 1) California - Accuri C6, 2) Canada - Galllicos and 3) Australia - FACSCanto. K2EDTA samples were collected from 71 voluntary participants in the Abbott donor program and spent specimens from subjects with normal CBC results and no abnormality expected to influence thrombopoiesis. Briefly: fresh (<8 hours old) whole blood was incubated for 15 mins in the dark with a CD61/41-APC mix and subsequently stained with TO before fixation with methanol-free formaldehyde. A second tube with PBS instead of TO served as the control. During analysis, a positive marker was drawn on an FL1 (TO) histogram (gated on the platelet cluster) of the control tube to include 0.1% of the negative population. The instrument settings and this marker were fixed for the acquisition and analysis of the tube containing the TO-stained platelets. Basic statistics were generated in MS Excel 2010, with Analysis of Variance used to assess mean results (male, female, pooled) across the 3 sites. Results: The mean RP results were: California 5.2% (SD 3.3%, n=21), Canada 4.8% (SD 0.7%, n=21) and Australia 4.6% (SD 3.0%, n=29). The mean for the 71 pooled subjects was 4.9% (SD 2.7%, Min 1.7%, Max 17.1%, Please see Figure 1). Differences in means between the 3 sites were not statistically significant (ANOVA, p = 0.769). Though females had a higher mean than males (6.1% versus 4.1%) the difference was also not significant (ANOVA, p = 0.174) Conclusions: Using a simple protocol we have shown that different operators, at different sites, with different instruments can generate comparable results in ‘apparently disease-free’ subjects. A shortcoming of nucleic acid based methods is non-specific dye uptake by platelet dense granules, resulting in no distinct positive population. We mitigated this by: 1) Titration of TO to a minimum dose, 2) Strict control of incubation conditions and 3) A simple, objective control strategy to help differentiate specific from non-specific staining. We believe this combination method is simple and robust enough to form the basis for a potential reference method. Further studies to characterize assay performance are planned.

608 ASSESSMENT OF IMMATURE PLATELET FRACTION AND IMMATURE RETICULOCYTE FRACTION AS PREDICTORS OF ENGRAFTMENT OF HEMATOPOIETIC STEM CELL TRANSPLANTATION

Iuri Vicente Morkis, Mariela Farias, Lisandra Rigoni, Luciana Scotti, Laura Gregiani, Liane Daudt, Lucia Silla, Alessandra Paz

Hospital de Clinicas de Porto Alegre Porto Alegre, Brazil

Introduction: Bone marrow engraftment has an important role in the hematopoietic stem cell transplantation (HSCT). Nowadays, engraftment criteria are absolute neutrophil count (ANC) above 0.5x10^9/L and platelet counts above 20x10^9/L, for three consecutive days. Others hematological parameters are being studied as early indicators of bone marrow recovery, like immature platelet fraction (IPF) and immature reticulocyte fraction (IRF). The aim of this study was evaluate these parameters as predictors of engraftment of HSCT. Methods: All patients submitted to HSCT at our Institution, during the period of 2013 March to September, were invited to participate. We evaluated IPF, IRF and hematological parameters daily, using the hematological analyzer Sysmex XE 5000 (Sysmex Corporation, Japan). IPF and IRF are measured by fluorescence and scatter light. Neutrophil recovery and platelet recovery were considered as the first of three consecutive counts above 0.5x10^9/L.
and 20x10^9/L, respectively, without transfusion support. We used IRF>12% as engraftment threshold, according to reference interval previously validated at our Institution. IPF>6,2% was a cut-off point of engraftment determined by reference interval, and IPF>10% was a cut-off point used in prior researches about IPF. We compared the day of neutrophil engraftment with first day of IRF>12%, and day of platelet engraftment with the first day of IPF>6,2% and IPF>10%. 

**Results:** Forty-four patient were enrolled in the study; twenty-four perform autologous HSCT and twenty perform allogeneic HSCT. IRF>12% anticipate neutrophil engraftment in 86% of patients (38/44), and IPF>6,2% anticipate platelet engraftment in 90% of patients (37/41). IPF>10% anticipate platelet engraftment in 63% of patients (26/41). Table 1 displays median day of engraftment and the statistical significance.

<table>
<thead>
<tr>
<th>Median day of</th>
<th>Autologous P</th>
<th>Allogeneic P</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANC engraftment</td>
<td>13.5 (10.25 – 15.0)</td>
<td>19.0 (14.25 – 22.5)</td>
</tr>
<tr>
<td>Platelet engraftment</td>
<td>11.0 (10.0 – 12.0)</td>
<td>19.0 (12.0 – 27.0)</td>
</tr>
<tr>
<td>IRF engraftment</td>
<td>9.5 (9.0 – 10.75)</td>
<td>&lt;0.001 13.0 (11.0 – 15.0) 0.006</td>
</tr>
<tr>
<td>IPF&gt;10% engraftment</td>
<td>9.0 (8.0 – 10.75) 0.011 15.0 (10.0 – 18.0) 0.004</td>
<td></td>
</tr>
<tr>
<td>IPF&gt;6% engraftment</td>
<td>9.0 (8.0 – 9.75) &lt;0.001 12.0 (7.5 – 15.0) 0.001</td>
<td></td>
</tr>
</tbody>
</table>

**Conclusions:** These results shows the potential of IRF and IPF as engraftment predictors, when they anticipate the rise in the absolute neutrophil count (ANC) and platelet count. Peak in IPF was observed before rise in platelet count, while IRF rises before ANC and persists elevated (fig.1). This indicates that both IRF and IPF can be considered as new tools for hematopoiesis assessment after HSCT.

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**610 A SIMPLE FUNCTIONAL ASSAY FOR HEPARIN-INDUCED THROMBOCYTOPENIA TESTING**

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1 Department of Hematology and Central Hematology Laboratory, Inselspital, Bern University Hospital and University of Bern Bern, Switzerland, 2 Department of Haematology, Auckland City Hospital Auckland, New Zealand, 3 Department of Molecular Medicine and Pathology, University of Auckland Auckland New Zealand

**Introduction:** Laboratory confirmation of Heparin Induced Thrombocytopenia (HIT) requires combined immunological and functional testing (J Throm Haemost 2011;9:2498). Current functional assays are time consuming and require special skills/equipment/facility. We developed a rapid functional assay, compared it with the tests currently used, and suggested a testing algorithm.

**Methods:** The Rapid Aggregation Test (RAT) relies on donor platelet count drop in a HIT+ plasma and heparin. Either 10 uL of normal saline, heparin 0.5 IU/mL or heparin 100 IU/mL was added to tubes containing 100 uL of donor platelet rich plasma and 50 uL of test plasma. The platelet counts of the tubes were measured after 15 minute incubation at 37°C with standard mixing. The platelet count in the heparin 0.5 IU/mL tube (test tube) was compared to the other two tubes. The RAT was done on 101 well characterised plasmas from patients with suspected HIT. The results were compared to the reference method, Heparin-Induced-Platelet-Aggregation (HIPAT), and ID-H/PF4-Particle-Gel-ImmunoAssay (PaGIA). Previous study showed a titre of 4 with PaGIA gave best balance between sensitivity and specificity for HIT detection (Haematologica 2012;97:89). 

**Results:** Figure 1 illustrates the platelet count change in typical HIT negative and positive cases. A >20% drop in the platelet count in the test tube compared to the controls was considered positive. The test took less than 30 minutes. The results of RAT on the 101 cases and comparison with HIPAT and PaGIA are shown in Table 1. Compared to HIPAT, RAT has a sensitivity of 78%, specificity 92%, Positive Predictive Value 84% and Negative Predictive Value 92% for HIT. RAT correlated well with PaGIA with no false positive at titre of <4.

<table>
<thead>
<tr>
<th>RAT+</th>
<th>RATneg</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>PaGIA titre</td>
<td>&lt;4</td>
<td>0</td>
</tr>
<tr>
<td>&gt;4</td>
<td>25</td>
<td>18</td>
</tr>
<tr>
<td>HIPAT</td>
<td>pos</td>
<td>21</td>
</tr>
<tr>
<td>neg</td>
<td>4</td>
<td>70</td>
</tr>
</tbody>
</table>

**Conclusions:** RAT performs well, can be done in any laboratory with a modern blood counter, and gives the result within 30 min. Combining the RAT and PaGIA improves the testing for HIT. Using a PaGIA titre of 4 as the cut-off, PaGIA+/-RAT+/-excludes all cases of HIT, and PaGIA+/-RAT+ picks up 80% of HIT+ cases. HIPAT is only required in discordant cases (PaGIA+/RATneg or PaGIA+/RAT+). This will allow rapid laboratory diagnosis of HIT which can be done in all clinical laboratories, and reduce significantly the need to perform the reference functional test.
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THE MOLECULAR-GENETICS MECHANISMS OF PLATELETS ACTIVATION IN PATIENTS WITH CEREBROVASCULAR DISEASE
Olga Sirotkina1,2,3, Anastasiya Laskovets1, Vitaliy Goldobin1, Tatiana Vavilova1,2,3
1B.P. Konstantinov Petersburg Nuclear Physics Institute Saint-Petersburg, Russia, 2Federal Almazov Medical Research Centre Saint-Petersburg, Russia, 3North-Western State Medical University named after I.I. Mechnikov Saint-Petersburg, Russia

Introduction: Cerebrovascular disease is a main cause of mortality and one of the great medical problems. After the vascular wall’s damage the endothelial cells secrete the von Willebrand factor which then connects with its platelet’s receptor GP Ib-V-IX. There are two polymorphisms Thr145Met and T(-5)C of the GP Ibα gene associated with arterial thrombosis development. On the other hand, circulating anucleate platelets, the progeny of megakaryocytes, retain a subset of pre-mRNAs and it is spliced into mature mRNA in response to cellular activation. The difference in platelets’ genes expressions was shown previously in patients with various clinical course of ischemic heart disease. The aim of this study was to investigate the role of platelet’s GPIba receptor in platelets’ activation in patients with cerebrovascular disease and analyze pre-mRNA and mRNA of GPIba gene in platelets of subjects with and without antiplatelet therapy. Methods: 123 patients (mean age 56±1) with cerebrovascular disease due to macroangiopathy (1 group), microangiopathy (2 group) and subjects with pathological tortuosity of brachiocephalic arteries (3 group) and 97 healthy donors (mean age 56±2, control group) were included into the study. We analyzed the level of GPIba receptors on platelet’s membrane by flow cytometry, Thr145Met and T(-5)C GPIba polymorphisms by PCR-RFLP, the GPIba gene expression by RT-PCR and ADP-induced platelet aggregation by Born method. Results: The distribution of T(-5)C GPIba genotype between patients and controls was not significantly different. However, the significant difference was observed in the Thr145Met GPIba genotypes’ frequencies: 73.2%, 22.0%, 4.8% and 89.4%, 10.6%, 0% for ThrThr, ThrMet and MetMet in 1 group and controls, respectively (p=0.045). Thereby, the presence of the 145Met GPIba allele in the homo- or heterozygous state was a risk factor for stroke due to macroangiopathy (OR=3.08[95%CI1.02-9.33]). The level of GPIba receptors on platelets’ membrane didn’t significantly differ in investigated groups. However the level of mature mRNA was lower in patients treated with acetylsaliclyc acid (ASA, 100mg/day) compared with subjects without antiplatelet therapy: 150±46 and 2268±1393, respectively (p=0.025), whereas pre-mRNA level didn’t differ in these groups: 13±6 and 20±12, respectively (p=0.07). The ADP-induced platelet aggregation also was lower in patients treated with ASA compared with non-treated: 77±5% vs 80±3% and 30±2%/min vs 37±2%/min (p=0.03; 10mkM ADP).

Conclusions: We have shown: 1) the Thr145Met GPIba influence atherotrombotic stroke development due to macroangiopathy; 2) the pre-mRNA transform into the mature mRNA in activated platelets and this process may be stopped by the antiplatelet therapy.

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STICKY PLATELET SYNDROME AND PLATELET EXPRESSION OF CD-62P
Ingrid Škoroňová1, Jana Fedorová3, Daniela Chuďá1, Ján Staško1, Pavol Holý2, Peter Kubisz1
1Comenius University, JLF Martin Martin, Slovakia, 2Hemo-Medika, s.r.o. Martin, Slovakia, 3National Transfusion Service SR s.r.o. Martin, Slovakia

Introduction: Inherited platelet hyperaggregability after low concentrations of platelet agonists adenosinediphosphate (ADP) and/or epinephrine (EPI) was reported as the sticky platelet syndrome (SPS). Clinical symptoms of SPS include unexplained arterial and venous thrombotic events, frequently under oral anticoagulant therapy and recurrent abortions. The aim of our study was to determine if there is any difference in platelet CD62P (P-selectin) expression between SPS patients and controls. Methods: Platelet membrane P-selectin expression was assessed by flow cytometry (FACScalibur, Becton Dickinson, USA) between SPS patients (n=100) and healthy blood donors (n=70). SPS was diagnosed using light transmission aggregometry employing the method by Mannen and Bick. Patients were divided in 4 groups, based on clinical manifestation: arterial thrombosis (AT), venous thromboembolism (VTE), abortions (AB) and asymptomatic individuals with SPS type II phenotype (AS). Man-Whitney’s U test was used for statistical analysis. Results: Platelets of the patients expressed significantly higher P-selectin levels compared to the controls 26.54 (15.85-45.32) vs. 19.11 (6.42-30.93) p<0.0001. Results in the groups compared to the controls were as follows: AT (N=19): 21.48 (14.33-42.17) p=0.055; VTE (N=28): 27 (15.88-47.86) p=0.06; AB (N=9): 27.63 (18.33-61.12) p<0.05 and AS (N=38): 28.9 (17.2-48.43) p<0.01, respectively. Conclusions: Platelets of patients with SPS type II expressed significantly higher P-selectin levels compared to controls, thus they seem to be activated even in asymptomatic individuals. Acknowledgement: This study was supported by project APVV 0222-11, grant Vega 1/0016/12, CEPV I (ITMS 26220120016) and CEVEPET (ITMS 26220120063) which are cofinanced from EC sources.

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IMPACT OF THE PROTON PUMP INHIBITORS ON PLATELET RESPONSE TO CLOPIDOGREL
A. Anil Timur1, Gurunathan Murugesan1, John Barnard3, Sanjay Gandhi3, Deepak L. Bhatt1, Kandice Kottke-Marchant1
1Cleveland Clinic, Robert J. Tomsich Pathology and Laboratory Medicine Institute Cleveland, OH, USA, 2Cleveland Clinic, Quantitative Health Sciences Cleveland, OH, USA, 3MetroHealth Campus-Case Western Reserve University, Heart and Vascular Center Cleveland, OH, USA, 4Brigham and Women’s Hospital, and Harvard Medical School Boston, MA, USA

Introduction: The interaction between clopidogrel and proton pump inhibitors (PPIs) has been suggested to result in poor platelet response to clopidogrel due to competitive inhibition...
Epidemiological, Clinical and Biological Characteristics of B-Thalassemia in Northwest of Algeria

Moulaeddoun Khedidja, Amin Moueden, Chakib Rahal, Khalida Berras, Fatima Homane, Naima Boubaker, Ramon Brouzet, José Carlos Almeida, Maria Leticia Ribeiro

Introduction: B thalassemia occurs when there is a quantitative reduction of beta globin chains that are usually structurally normal. Their high frequencies constitute a real public health problem in Algeria from the economical and human aspects.

Methods: Our study was conducted at the level of the unit of Biochemistry-Hematology at the hemobiology service CHU-O oran: it was conducted in two stages: A retrospective study (from May 2011 to March 2013) and a prospective one (from March to August 2013), it offered a better understanding of thalassemia in our region by describing their epidemiological aspects, so we followed the next diagnostic procedures: a detailed questionnaire, a CBC complete blood count by the medical instrument (Medonic®), a peripheral blood smear, an iron status (the determination of iron and total iron-binding capacity), capillary electrophoresis of the Hb by (CAPILLARYS® Sebia) and a family survey at the end to confirm the diagnosis. Results: We obtained the following results: 33.3% of patients were below 10 years old. Inbreeding was involved in 30.5% of the cases. 66.67% of the haemoglobinopathies were detected following a family survey.

Aim: Develop easy to use diagnostic tool for screening HS based on hematological parameters used in our routine analyzer ABX HOBIRA Pentra DX 120, like the reticulocyte counts and the immature reticulocytes fraction. Methods: Peripheral blood samples (n=150) processed on EDTAK3 in analyzer Pentra DX 120, levels of RET# and IRF determined from a cohort of 25 confirmed HS. Population divided in groups: Autoimmune hemolytic anemia(AIHA) = 15; iron deficiency anemia (IDA) = 30; Intermedia Beta Thalassemia (IBThal) = 8; Cord blood samples (CB) = 30; healthy subjects (control) = 42 and then compared with HS group. Statistical analysis, of RET#, IRF, RETH#, IMM# and ROC curve analysis, for the sensitivity, specificity values, using GraphPad Prism 5.

Results: Median for each group: Control (RET#:48.5; RETH#:1.7; RETM#: 7.5; IMM#: 0.03); AIHA (RET#: 220; RETH#:12; RETM#: IMM#: 0.01); IDA (RET#: 50; RETH#: 2.1; RETM#: 8.3; IMM#: 0.03) HS (RET#<299; RETH#<0.7; RETM#: 5.8; IMM#: 0.04); IBThal (RET#: 215; RETH#: 11.2; RETM#: 22.6; IMM#: 0.79); CB (RET#: 187; RETH#: 7.5; RETM#: 20.35; IMM#: 0.1). ROC curve analysis: sensitivity 100% and 99.91% specificity for RET#<IRF < 10 to HS. Conclusions: Has waiting result HS had high RET# count (median=299) without a matching elevated IRF (median=12.8). RET#<IRF cut-off was >10 with excellent sensitivity and specificity. Observed increased IMM in the IBThal group due to increased NRB. Despite increased IMM the RET in the cord blood is shortened. This simple and fast screening method could be used like a rule for trial HS using the HOBIRA ABX.
PENTRA Hematological instruments. However the HS diagnostic still have to be confirmed by more sensitive and accurate tests such as cryohemolysis, eosin-5’-maleimide (EMA) binding test and membrane proteins electrophoresis.

704 VALIDATION OF THE CUTOFF VALUES OF THE MEAN CORPUSCULAR VOLUME AND MEAN CORPUSCULAR HEMOGLOBIN ON FOUR AUTOMATED ANALYZERS FOR THALASSEMIA SCREENING
Chanthorn Chatiriporn1, Kanokwan Sanchaisuriya2, Sakoun Inthavong3, Supan Fuchareon2, Goonnapa Fuchareon2, Yossombut Changtrakul4, Pattara Sanchaisuriya2
1Medical Science Program, Graduate School, Khon Kaen University Khon Kaen, Thailand, 2Centre for Research and Development of Medical Diagnostic Laboratories (CMDL), Faculty of Associated Medical Sciences, Khon Kaen University Khon Kaen, Thailand, 3Centre for Research and Development of Medical Diagnostic Laboratories (CMDL), Faculty of Associated Medical Sciences, Khon Kaen University Khon Kaen, Thailand, 4Clinical Microscopy unit, Srinagarind Hospital, Faculty of Medicine, Khon Kaen University Khon Kaen, Thailand, 5Department of Nutrition Faculty of Public Health, Khon Kaen University Khon Kaen, Thailand

Introduction: The combined dichlorophenolindophenol (DCIP) test and mean corpuscular volume (MCV) or mean corpuscular hemoglobin for (MCH) has been recommended for use in a primary screening for alpha-(0)-thallassemia, beta-thallassemia and hemoglobin E (Hb E) in Southeast Asian populations. For MCV, the cutoff values of 78 fl and 80 fl have been proposed. For MCH, the generally used cutoff value is 27 pg. In this study, we validated the effectiveness thalassemia screening using the DCIP test in combination with either MCV or MCH obtained from 4 hematology analyzers. Methods: Blood samples from 325 Laotian students who enrolled the screening program for thalassemia were used. The MCV and MCH values were measured using 4 models of hematology analyzers. Hb E carriers were initially screened by the KOU-DCIP test. The diagnosis of alpha-(0)-thalassemia, beta-thalassemia and hemoglobin E (Hb E) was made using hemoglobin and DNA analyses. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated to determine the effectiveness of the combined test. Four cutoff values, i.e. MCV < 80 fl, MCV < 78 fl, MCH < 27 pg, and MCH < 25 pg, in combination with the DCIP were validated. Results: Of the 325 participants, 134 (41.2%) were found to be the target carriers, i.e. 21 (6.5%) alpha-(0)-thalassemia, 5 (1.5%) beta-thalassemia, 102 (31.4%) Hb E, and 6 (1.8%) individuals with complex interaction of alpha-(0)-thalassemia and Hb E or beta-thalassemia. The combined DCIP and MCV or MCH obtained from the 4 hematology analyzers provided 100% sensitivity and 100% NPV. With different cutoff values, the specificity of the combined test using 4 hematology analyzers varied considerably from 52 - 81%. Among those cutoff values, the combination of the DCIP with MCH < 27 pg provided highest false positive rates resulting in a poor specificity of 52-57%. However, when the DCIP test was used in combination with either MCV < 78 fl or MCH < 25 pg, the highest specificity of 74-80% was obtained. Conclusions: Using the 4 hematology analyzers tested, MCV < 78 fl and MCH < 25 pg appear to be the appropriate cutoffs. Either of these values in combination with the DCIP test could be used effectively for thalassemia screening in an area prevalent for alpha-(0)-thalassemia, beta-thalassemia, and Hb E.

706 EFFECT OF BLOOD STORAGE ON OSMOTIC FRAGILITY
Diweni Dick, Teddy Adias, Andrew Etima, Victor Ideede, Ayibatonye Orutugu, Idaye Reuben, Mercy Igoni, Christian Atiegha
Bayelsa State College of Health Tech. Yenagoa, Nigeria

Introduction: Blood storage is an integral part of immunohaematology, especially in transfusion medicine. This study aims at determining the effect of storage on osmotic fragility. Methods: Colorimetric method was used for osmotic fragility analysis on 5 units of blood, obtained from apparently healthy relative donors. Results: Results reveal that blood stored in blood bank refrigerator for transfusion at 4°C with CPDA-1 maintained an insignificant variability for 35 days (P > 0.05) and a mean corpuscular frequency (MCF) value of 4.4 – 5.5 x 10^11 while the blood sample stored after 35 days to 42 days showed a significant increase (P < 0.05) in MCF value of 6.7 x 10^11. Table A: Comparison Analysis on Osmotic Fragility from Baseline (0 day) to 42 days

<table>
<thead>
<tr>
<th>% Haemolysis (mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline (0 day)</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>7</td>
</tr>
<tr>
<td>14</td>
</tr>
<tr>
<td>21</td>
</tr>
<tr>
<td>28</td>
</tr>
<tr>
<td>35</td>
</tr>
<tr>
<td>42</td>
</tr>
<tr>
<td>β Baseline (0 day) indicate control (before storage) *No significant differences observed (P &gt; 0.05) using ANOVA</td>
</tr>
</tbody>
</table>

Table B: Comparison Analysis on Osmotic Fragility from 2% PBS (Phosphate Buffered Saline) to 8% PBS

<table>
<thead>
<tr>
<th>%PBS</th>
<th>% Haemolysis (mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>96.11 ± 0.54</td>
</tr>
<tr>
<td>3</td>
<td>93.67 ± 0.62</td>
</tr>
<tr>
<td>3.5</td>
<td>91.00 ± 0.41</td>
</tr>
<tr>
<td>4</td>
<td>83.00 ± 1.64</td>
</tr>
<tr>
<td>4.5</td>
<td>61.22 ± 4.42</td>
</tr>
<tr>
<td>5</td>
<td>40.11 ± 7.10</td>
</tr>
<tr>
<td>6.5</td>
<td>17.33 ± 6.37</td>
</tr>
<tr>
<td>7</td>
<td>8.55 ± 3.13</td>
</tr>
<tr>
<td>8</td>
<td>3.67 ± 1.55</td>
</tr>
<tr>
<td>β Baseline (2% PBS) indicate control (before storage) *Significant differences was observed (P &lt; 0.05) using ANOVA</td>
<td></td>
</tr>
</tbody>
</table>

Conclusions: It was recommended that blood stored at 4°C with CPDA-1 anticoagulant should not be transfused after 35 days due to increase in osmotic fragility of the red blood cells which brings about haemolysis due to prolonged storage.
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CASE STUDY: BETA THALASSEMAIA MAJOR WITH AN UNDERLYING APOLIPOPROTEIN A5 MUTATION.

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Introduction: Patient AS, a 6 month old male, whose family is from India, was taken to a local medical clinic due to “looking a bit pale”. Mother was known to be microcytic. Methods: Patient bloods were collected and sent for analysis. The most striking feature was the appearance of the plasma, being grossly lipemic. Haemoglobin was corrected by both plasma replacement and by blank correction methods. Strong centrifugation (15000rpm for 30 minutes) was used to separate and subsequently remove chyloimicron layer from plasma to reduce lipid interference of chemistry analysis, where appropriate. Results shown in Table 1.

Results:

Table 1 - Patient Results

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Reference Range</th>
<th>Day 1</th>
<th>Day 3</th>
<th>Day 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum Appearance</td>
<td>Lipaemic</td>
<td>Lip. +++</td>
<td>Lip. ++</td>
<td></td>
</tr>
<tr>
<td>Triglyceride</td>
<td>&lt; 1.7 mmol/L</td>
<td>50.2</td>
<td>17.5</td>
<td>2.47</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>&lt;4.5 mmol/L</td>
<td>9.16</td>
<td>6.75</td>
<td>5.46</td>
</tr>
<tr>
<td>Haemoglobin</td>
<td>100-140 g/L</td>
<td>65</td>
<td>83</td>
<td>78</td>
</tr>
<tr>
<td>MCV</td>
<td>78-90 fl.</td>
<td>68</td>
<td>70</td>
<td>72</td>
</tr>
</tbody>
</table>

The presence of numerous red cell fragments and nucleated red cells suggested a beta thal major – Heinz body staining was negative. Subsequent testing showed both parents to be heterozygous for beta thalassaemia mutations and the patient a double heterozygote mutation (Table 2). Although hypertriglyceridaemia is well described in beta thalassaemia major, cholesterol is generally described as low, so the hypercholesterolaemia was unexpected. Also, the triglyceride is well above literature values for beta thalassaemia major. Further testing showed an underlying Apolipoprotein A5 mutation, and that this was the primary cause of the hyperlipidaemia.

Table 2 - Subsequent Testing: Patient A5

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Reference Range</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>HbA2</td>
<td>1.5-3.5%</td>
<td>5.0%</td>
</tr>
<tr>
<td>HbF</td>
<td>&lt;5%</td>
<td>93%</td>
</tr>
</tbody>
</table>

DNA Analysis: Heterozygous for ApoA5 Variant

Conclusions: Patients with APOA5 mutation may have ongoing health issues, including cardiac problems, so it is important to diagnose and appropriately treat affected individuals, particularly if underlying another chronic condition such as Beta Thalassaemia Major which may also have cardiac problems from multiple transfusions.

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DIFFERENTIATION OF HB E-DB+THALASSEMAIA AND HB E-B+THALASSEMAIA BY HB-ANALYSIS USING CAPILLARY ELECTROPHORESIS

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Introduction: dbδ-Thalassemia is a genetic condition associated with high expression of Hb F in adult life. Interaction of dbδ-thalassemia with Hb E leads to similar hematological features with that observed in Hb E-b-thalassemia but milder clinical feature. Differentiation between the two conditions is essential and usually requires molecular testing. We described a simple differentiation of these two conditions. Methods: Study was done on adult subjects with dbδ-thalassemia/Hb E (n=21), b-thalassemia/Hb E (n=45) and b-thalassemia/Hb E (n=29) diseases. Hematological parameters were recorded on Coulter T series (Beckman-Coulter Co., CA., USA) and Hb analysis was done on capillary electrophoresis (Capillaries 2 Flex Piecing; Sebia, Lisses, France). DNA deletions causing δβ-thalassemia and b-thalassemia were identified using PCR assays. Results: DNA analysis identified the 12.6 kb δβ-thalassemia deletion in trans to the Hb E gene in all 21 cases of dbδ-thalassemia/Hb E. Mutations causing bδ and b-thalassemias were identified in the remaining cases. Hematological features demonstrated that Hb E-dbδ-thalassemia is associated with a relatively milder phenotype with Hb 12.3±2.0 g/dL. These were found to be 7.5±1.5 and 10.3±1.8 g/dL for Hb E-bδ-thalassemia and Hb E-b-thalassemia, respectively. Hb analysis identified 2.2±0.3% Hb Aα, 42.0±2.8% Hb E and 54.0±4.3% Hb F in Hb E-dbδ-thalassemia whereas these were 6.1±1.9% Hb Aα, 57.2±13.0% Hb E & 34.8±15.1% Hb F and 7.1±2.2% Hb Aα, 52.8±6.5% Hb E & 15.6±10.0% Hb F for Hb E-bδ-thalassemia and Hb E-b-thalassemia, respectively. Conclusions: Although with similar hematological findings, Hb E-dbδ-thalassemia had relatively milder phenotype than Hb E-b-thalassemia. The normal Hb Aα with higher Hb E than Hb F levels are useful markers for initial recognition of case with Hb E-dbδ-thalassemia and differentiation of the two conditions.

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PRIMARY PREVENTION OF HEMOGLOBINOPATHIES BY PRENATAL DIAGNOSIS AND SELECTIVE PREGNANCY TERMINATION IN A MUSLIM COUNTRY: OMAN

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1Hemoglobinopathies Laboratory, dept. of Clinical Genetics, Leiden University Medical Center Leiden, Netherlands, 2Molecular Genetic Laboratory, National Genetics Centre Muscat, Oman

Introduction: Haemoglobinopathies (HBP) are the most common genetic disorder in Oman and are in need of prevention. Prenatal diagnosis (PD) and selective pregnancy termination is shown to be the most effective prevention tools for the control of HBP. However, PD is not implemented in Oman thus far because abortion is subject to religious values and appeared to be accepted by 70% only if it was religiously allowed. Methods: Results: Conclusions:
Introduction: Given the resolution/sensitivity of modern mass spectrometers we explore the possibility to differentiate and quantify intact proteins. Rather than the analysis of small molecules or protein digestives, we refrain from any sample preparation and analyze hemoglobin by direct injection of lysed RBC’s in an ESI-Q-TOF setup. We compare results obtained by HPLC of healthy patients and subjects with hemoglobinopathies and thalassemias.

Methods: A 5-step optimization study was performed for appropriate conditions in buffering, injection, ionization, mass acquisition and spectral deconvolution. The mass distributions of the a- and b-chain and the a/b-intensity ratio were deduced from a population comprising of 50 donors serving as a reference. After HPLC measurements, patient samples were analyzed by MS, in which the a/b-intensity ratio was used as a screening parameter. Deviations herein were rationalized by the presence of other peaks than aand b(hemoglobin variant, confirmed by HPLC) or low abundance of a- or b globulins (thalassemia, confirmed by HPLC and DNA).

Results: Unlike the within-run precision, an EP-15 study revealed a poor within-lab precision for the a/b-intensity ratio, while narrow mass distributions were obtained in any case for aand b. Normal distributions were found for the a/b-masses and the a/b-intensity ratio of the donor population, yet the resulting reference did not match the patient population due to the poor precision. Mass deviations from patient samples outside the reference interval revealed the presence of different hemoglobin variants as confirmed by HPLC.

Conclusions: The mass resolution of this modern ESI-Q-TOF setup is sufficient to differentiate globulin proteins with mass differences up to 1 Da. However, quantitative measurements by the a/b-intensity ratio remain difficult; investigations after ion suppression, sample contamination and the possible need for an internal standard are underway.

Introduction: Glucose-6-phosphate dehydrogenase (G6PD) deficiency is one of the most common human enzyme deficiencies in the world. It is particularly common in populations living in malaria-endemic areas, affecting more than 400 million people worldwide. This present study was conducted with the aim of determining the prevalence of G6PD deficiency among children visiting the Emergency Paediatric Unit of Usmanu Danfodiyo University Teaching Hospital for pediatric-related care. The study included 118 children, made up of 77 (65.3%) males and 41 (34.7%) females aged #5 years with mean age of 3.26 ± 1.90 years.

Methods: Randox G6PD quantit-ive in vitro test screening was used for the diagnosis of G6PD deficiency according to the manufacturers’ instructions. Results: Of the 118 children tested, 17 (14.4%) were G6PD-deficient. Prevalence of G6PD deficiency was concentrated predominantly among male children (22.1%). Male sex was significantly correlated with G6PD deficiency among the children studied (r = 7.85, P = 0.01). The highest prevalence occurred among children in the 2- to 5-year age-group. Of the 17 G6PD-deficient children, twelve (70.2%) were moderately deficient, while five (29.4%) were severely deficient. Blood film from G6PD-deficient children indicated the following morphological changes; Heinz bodies, schistocytes, target cells, nucleated red cells, spherocytes, and polychromasia. Table 1 Distribution of G6PD deficiency based on sex Sex Number (%) Number (%) G6PD-deficient(2.9 U/gHb) Male 77 (65.3) 17 (22.1) Female 41 (34.7) 0 (0.0) Total 118 (100.00) 17 (14.4)

Conclusions: This present study has shown a high prevalence of G6PD deficiency among children residing in Sokoto in the northwestern geopolitical zone of Nigeria. The study indicated a male sex bias in the prevalence of G6PD deficiency among the children studied. There is a need for the routine screening of children for G6PD deficiency in our environment, to allow for evidence-based management of these children and to ensure the avoidance of food, drugs, and infective agents that can potentially predispose these children to oxidative stress as well as diseases that deplete micronutrients that protect against oxidative stress. There is need to build capacity in our setting among pediatricians to ensure the effective management of children with G6PD deficiency.

Introduction: Laboratory diagnosis of hereditary spherocytosis (HS) is based on screening and confirmatory tests; our working algorithm includes morphology analysis, cryohaemolysis, and eosine-5-maleimide binding test as screening tests, and sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) as diagnostic test. Another well-known confirmatory test, the ektacytometry, analyses the deformability of red blood cells (RBC) in changing osmotic environment with applied constant shear stress. The LoRRca MaxSis (Mechatronics Instruments, The Netherlands) is a new generation ektacytometer that has recently appeared on the market. It is a fully automated laser-assisted optical rotational cell analyser, capable to measure only for 10 minutes the elongation index (EI) of RBC as a continuous function of suspending medium osmolality. An osmocan curve (figure 1) with the following parameters of interest is thus obtained: O min (the osmolality giving the minimal EI), El max (the maximum EI), and the area under the curve (AUC).

Methods: We realised an in site validation of LoRRca by analysing samples (n=110) from 5 control subjects. The blood samples were K2-EDTA or ACD (acid-citrate dextrose) anticoagulated and kept at 4°C or at 20°C for 7 days. During a period of six months, we performed, in parallel with the SDS-PAGE, the osmoscan analysis of samples from patients with the following RBC membrane pathologies: HS (n = 21), autoimmune haemolytic anaemia (n = 5), hereditary elliptocytosis (n = 1), and congenital dyserythropoietic anaemia (n = 1).

Results: The
Inter-assay variability was lower than 0.03%. The stability of the evaluated parameters observed for different conservation modes and delays of blood samples is presented on table I. The osmoscan curve analysis, EDTA samples are stable during 72h if refrigerated and ACD samples are stable during 72h at room temperature and up to one week if refrigerated. The LoRrca MaxSis instrument could be successfully implemented for rapid automated HS diagnosis in specialized red cell analysis laboratories.

720 HEREDITARY DEFICIENCY OF PYRUVATE KINASE (PKLR) ASSOCIATED WITH CHRONIC HAEMOLYSIS. CONTRIBUTION OF PKLR GENE SEQUENCING TO DIAGNOSIS IN CASES OF HIGH RETICULOCYTOSES

Laura Montllor, Maria del Manuñ Pereira, Pilar Goméz, Jessica Abad, Laura Olaya, Juan L Luis Vives Corrons

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Introduction: Pyruvate kinase (PK; EC2.7.1.40) is a key enzyme of anaerobic glycolysis encoded by the gene PKLR. PK deficiency is a cause of chronic non-spherocytic hemolytic anemia (CNHA) affecting homozygous or double heterozygous conditions of PK gene mutation. Over 240 mutations of PKLR mutations have been so far described [www.lovd.nl/PKLR]. The diagnosis of PK deficiency is based on the measurement of enzyme activity in the hemolysate, although the results can be masked by an increased RBC young populations (reticulocytes) or by the effect of transfusion. To avoid these effects the PK/HK ratio can be used. Since HK activity is another age dependent RBC enzyme, in PK deficiency it is two or three fold higher than PK activity. Accordingly, in PK deficiency the PK/HK ratio is significantly decreased and the diagnosis can be confirmed by sequencing PKLR gene. We report here the result of sequencing in patients with CNHA referred to our Unit for diagnosis. Methods: We studied 10 patients aged 9 months to 37 years whose PK activity was determined from EDTA anticoagulated venous blood by the procedure standardized by ICSH. Gene sequencing has been performed on PKLR 20 alleles by conventional Sanger method using ABI Prism 373A GeneticAnalyzer (Applied Biosystems PE).

Results: In all cases the value of the PK was below the reference range (8.4-15.2 IU/ gHB) 4 near the lower limit (6.60, 6.61, 6.76, 7.2) cases and in 4 clearly decreased (4.04, 4.4, 4.7, 5.56) and in 2 markedly decreased (1.35, 2.19) Genetic study demonstrated the high incidence of c.1456 C > T (4 cases) and c.721 G > T (5 cases) mutations as previously described in Spain, two deletions (c.315/316delG and c.203/204delC), one mutation in the promoter region ( - 142G > T) and one of the 3 untranslated (c.1738T > C). The other 8 other mutations, already described, were: c.1529G > A, c.359C > T, c.1168G > A, c.994G > A, c.1010G > A, c.1116+2 T > G and c.1618+1G > A. Only one allele showed no mutations. Conclusions: The genetic study of PKLR is essential for confirmation for PK deficiency in patients CNHA with decreased PK activity of the haemolysate. But it is especially useful when PK deficiency is masked by reticulocytosis or by the effect of transfusions. Also it allows the genetic counseling of affected families.

722 THE CHALLENGES OF HAEMOGLOBINOPATHY SCREENING IN A MULTIETHNIC POPULATION

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Introduction: Despite our extensive knowledge of the hematological and molecular changes in haemoglobinopathies, full blood count (FBC) and high performance liquid chromatography (HPLC) continue to be the main tools of screening. In Australia, where multiethnic communities are the norm, this provides ongoing challenges to our hematology services as we try to detect and characterize complex hemoglobinopathies. This is particularly important in antenatal care so that couples are aware of the risk of conceiving a child with a severe haemoglobinopathy. Methods: We reviewed two databases of haemoglobinopathy screening that were performed in our laboratory between 1996 and 2013. All testing in that period involved a FBC, HPLC, hemoglobin H inclusion body staining as well as alkali and acid gels. Database 1 was created in January 1996, and was stopped in November 2005 due to a change in laboratory information systems. During this period, screening was selective and aimed at high risk populations. Database 2 was created in January 2007 with analysis carried out at the end of June 2013. During this time period universal antenatal screening was introduced in recognition of our high risk population. We compared the demographics and range of haemoglobinopathy results in each database focusing on inconclusive results.
Results:

<table>
<thead>
<tr>
<th></th>
<th>Database 1</th>
<th>Database 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>No of patients</td>
<td>8,721</td>
<td>12,614</td>
</tr>
<tr>
<td>Abnormal results identified, n(%)</td>
<td>3,238 (37%)</td>
<td>1,758 (14%)</td>
</tr>
<tr>
<td>Most common abnormalities</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beta thalassaemia variants</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alpha thalassaemia variants</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Haemoglobin E trait</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inconclusive results</td>
<td>10%</td>
<td>17%</td>
</tr>
<tr>
<td>Number of different abnormalities</td>
<td>35</td>
<td>39</td>
</tr>
</tbody>
</table>

From a multiethnic perspective, database 2 had country of origin data on over 60% of patients with over 140 different countries represented. Inconclusive results were largely represented by the antenatal population where iron status was not provided and single chain alpha deletions could not be excluded. **Conclusions:** In our review of our hemoglobinopathy screening we identified that we have an inconclusiveness rate of 17%. The high number of inconclusive results is because the standard methods of analyses (FBC, HPLC, etc) are unable to exclude single and double gene alpha chain deletions. Thus multiplex polymerase chain reaction analysis of the alpha globin complex performed in a timely fashion may be warranted to provide accurate antenatal information to patients and their families.

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AN INVESTIGATION OF THE PROTECTIVE EFFECT OF ALPHA+-THALASSAEMIA AGAINST SEVERE PLASMODIUM FALCIPARUM AMONGST CHILDREN IN KUMASI, GHANA

Clement Opoku-Okrah 1, Michael Gordge 2, Emmanuel Kweku Nakua 2, Tsiri Agbenyega 2, Martin Parry 2, Claire Robertson 2, Caroline Smith 2

1Kwame Nkrumah University of Sci and Tech Kumasi, Ghana, 2University of Westminster London, United Kingdom

**Introduction:** Several factors influence the severity of Plasmodium falciparum; here, we investigate the impact of alpha+-thalassaemia genotype on P. falciparum parasitemia and prevalence of severe anaemia amongst microcytic children from Kumasi, Ghana. **Methods:** Seven hundred and thirty-two children (≤10 years) with P. falciparum were categorised into normocytic and microcytic (mean cell volume ≤76 fL). Microcytic individuals were genotyped for the α-thalassemia mutation and parasite densities determined. **Results:** Amongst microcytic patients both parasite densities and prevalence of severe malaria parasitemia (≥100 000/μL) were significantly lower (P < 0.001) in the presence of an alpha+-thalassaemia genotype compared with non-alpha+-thalassaemia genotype. There was no evidence that alpha+-thalassaemia protected against severe anaemia. **Conclusions:** The severity of P. falciparum parasitemia was significantly lower in both the homozygous and heterozygous alpha+-thalassaemia groups compared with microcytic individuals with non-alpha+-thalassaemia genotype. The protective effect, from severe malaria of the alpha+-thalassaemia allele does not alter with age.

Table 1. Prevalence of severe Malaria parasitaemia (defined as >100 000/μL) and severe anaemia (defined as Hb < 5 g/dL) in patients with different alpha thalassaemia genotypes

<table>
<thead>
<tr>
<th></th>
<th>Normocytic</th>
<th>Microcytic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mps &gt;100 000/μL Severe anaemia (Hb &lt; 5 g/dL), n (%)</td>
<td>358 (19.2%)(32)</td>
<td>126 (10.9%)(12.6)</td>
</tr>
<tr>
<td>Alpha thal genotype not determined</td>
<td>143 (34.7%) (32.9)</td>
<td>49 (8%)(18.4)</td>
</tr>
<tr>
<td>Non Alpha thal genotype</td>
<td>358 (19.2%) (32)</td>
<td>126 (10.9%) (12.6)</td>
</tr>
<tr>
<td>Alpha thal heterozygotes</td>
<td>192 (53%) (32.9)</td>
<td>7 (5.6)</td>
</tr>
<tr>
<td>Alpha thal homozygotes</td>
<td>8 (16.3%)</td>
<td>9</td>
</tr>
</tbody>
</table>

*P < 0.001 by chi-squared analysis vs. normocytic and microcytic with non-alpha thal genotype

Figure 1: Alpha+-thalassaemia genotype protects against severe Plasmodium falciparum parasitemia in children both less than and greater than 60 months of age. For each age group within each alpha-thalassemia genotype the percentage of children with moderate (1–9999), high (10 000–99 999) and severe malaria (>100 000) has been calculated.
726 EVALUATION OF THE MINDRAY BC-6800 AND BC-3600 AUTOMATED HEMATOLOGY ANALYZERS FOR SCREENING OF THALASSEMIA IN SOUTHEAST ASIAN POPULATIONS

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Introduction: Thalassemia is a most common genetic disorder worldwide including Southeast Asia. The patients have chronic anemia and need regular blood transfusion and iron chelation creating economic burdens for families and the nation’s health care. Thus the prevention of birth of severe thalassemia is crucial for many countries with high incidence of the abnormal genes. Thalassemia carrier screening in combination with genetic counseling and prenatal diagnosis with selective abortion is needed for the prevention of birth of severe thalassemia. Red cell indices (RI) have been widely used as primary screening for thalassemia carriers. It can be done by automated blood cell analyzer. However, quality control and standardization for those machines are very crucial and each lab use different cut off points for their screening. In this study, we evaluated two automated blood cell analyzers BC-6800 (a high end 5-PD analyzer with a 3D analysis tool especially created for this study) and BC-3600 (a top line 3-PD analyzer) from Mindray (Nanshan, Shenzhen, China) and compared for RI in normal and Thalassemia cases against our currently used test systems as reference. Methods: 312 blood specimens at Thalassemia Research Center, Mahidol University included 56 normal and 256 thalassemia and hemoglobinopathies were used for measurement RI. All blood specimens were collected using EDTA as anticoagulant. RI were measured on Mindray BC-6800 and BC-3600. Serum ferritin was used to evaluate the iron status of all subjects. Hemoglobin analysis was done using an automated HPLC and a- and b-thalassemia genotypes were determined using GAP PCR and reverse dot blot. Results: The RI results obtained from both analyzers confirmed that thalassemia ‘traits’ (heterozygotes) have low MCV and MCH while thalassemia disease (homozygotes) also exhibited low hemoglobin (Hb), hematocrit (Hct), and high red blood cell distribution width (RDW-CV%). The cut-off values of MCV for thalassemia trait by Mindray BC-6800 and BC-3600 were 83.3 and 83.9 fl, and cut off values of MCH were 27.6 and 28.1 pg, respectively. Results of reticulocytes, platelets and NRBCs were all increased in splenectomized beta-thalassemia/HbE patients when compared to that of non-splenectomized beta-thalassemia/HbE: patients and the 3D analysis tool provided on BC-6800 analyzer showed promising utility and our observations will be subject of separate study. Conclusions: Our results demonstrated diagnostic utility of RI data provided by both BC-6800 & BC-3600 analyzers in both normal and thalassemia disorders against our current test systems held as reference.

728 MATHEMATICAL MODEL-BASED ESTIMATION OF RED BLOOD CELL CLEARANCE PREDICTS FUTURE ANEMIA IN PATIENTS WITH NORMAL COMPLETE BLOOD COUNTS

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Introduction: The healthy human hematologic system is held in a state of dynamic equilibrium by the carefully regulated processes of (1) cell production, (2) cell maturation in the peripheral circulation, and (3) cell clearance. The resulting steady state is routinely quantified by such measurements as hematocrit (HCT), hemoglobin (HGB), or red blood cell count (RBC). Diseases perturbing one of these processes may trigger compensation in the others, maintaining the steady state -- and confounding our ability to diagnose or monitor the underlying diseases. Estimates of the rates of the underlying processes would serve as more sensitive biomarkers of disease, and the reticulocyte count, which provides a crude estimate of cell production, can help speed the identification of some pathologic conditions. An estimate of RBC clearance would likely provide similarly useful and complementary diagnostic information. Methods: We used a mathematical model of RBC population dynamics1 to infer RBC maturation and clearance rates for a large set of patients from routine CBC and reticulocyte counts performed on an Abbott CELL-DYN Sapphire automated hematology analyzer. We then assessed the diagnostic efficiency of estimated clearance threshold predicting future mild anemia in a subset of patients with normal CBCs. Results: We found that the estimated RBC clearance threshold is more tightly regulated in a healthy population (600 individuals) than existing CBC indices (Table 1). We then estimated rates of cell clearance in a retrospective study of patients with normal CBCs who then develop mild anemia. We identified 40% of these future anemic patients at the time of an entirely normal CBC up to 6 months prior to their presentation with anemia. Conclusions: Anemia is an early sign of a wide range of underlying diseases, such as iron deficiency, gastrointestinal cancer, and leukemia. Our results suggest that RBC clearance threshold is often reduced possibly as compensation for diminished erythropoietic output associated with these conditions. This compensation confounds our ability to detect the illness by measuring HCT or HGB. But by using a mathematical model of in vivo RBC population dynamics, we can estimate a patient’s clearance threshold and identify 40% of patients who go on to develop anemia in the subsequent 6 months. Existing diagnostic approaches identify 0% of these patients because their CBCs are entirely normal at the time of analysis.

730 AN INNOVATIVE APPROACH FOR DETECTING RED BLOOD CELL AGGLUTINATIONS WITH BC-6800 HEMATOLOGY ANALYZERS

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Introduction: Red blood cell (RBC) agglutination in patient blood has both clinical implication and laboratory significance. Cold
agglutination disease is an autoimmune disorder where red blood cells are bound by circulating antibodies, activating the pathway of the complement system, which may further lead to hemolytic anemia. Therefore detecting RBC agglutination in patient blood is very useful in diagnosing the cold agglutination diseases. Moreover, since samples with RBC agglutination would be reported with erroneous results on parameters such as RBC, MCV, HCT and MCHC, alerting users for its presence is critical for reliable and accurate laboratory hematology reports. Previous studies suggested using the value of HCT, HGB, MCHC and their differences from the normal ranges as indicators for detection of red blood cell agglutination. But its accuracy is very limited due to the fact that these parameters have wide range of biological variations. Both false alarm and mis-identification can happen quite frequently. In the current study, a novel approach based on mathematical modeling was developed and tested. The performance is significantly better than the traditional method. **Methods:** The RBC histograms from hematology analyzers have secondary populations to the right of the main one, and they are usually referred to as the “tails” of RBC histograms. Using Poisson statistical modeling, the distributions of the “tails” for normal samples can be well calculated and they match perfectly with the actual data. However, in samples with red cell agglutination, the “tails” from actual data are significantly higher than the mathematically calculated ones. The difference of the two is a good indicator of RBC agglutination, an unnatural distribution of red blood cells. **Results:** 12 samples with different levels of in-vitro introduced agglutination were initially tested. If the true positive judgment threshold is set as “RBC# reduces by 10% or 0.2 *1012/L”, 5 samples were negative and 7 positive. Testing results show that all 12 samples were correctly identified, while the traditional method mis-classified 6 out of 12 samples. 2 clinical samples with red cell agglutinations were available for testing our statistical model. Both patient samples were correctly identified with the proposed methods, while the traditional method could detect only one of them. **Conclusions:** Based on the preliminary testing results, the proposed method outperforms the traditional method and can be reliable in alerting users for the presence of red cell agglutination accurately.

732 ELECTROPHEROGRAM PATTERNS OF HB CONSTANT SPRING AND HB PAKSE ON CAPILLARY ZONE ELECTROPHORESIS

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**Introduction:** Interaction of Hb CS or Hb PS with α-thalassemia is a cause of Hb H disease. Hb CS or Hb PS is reported in hemoglobin analysis when there is a minipeak at zone 2 on electropherogram of capillary zone electrophoresis. Shift of this minipeak to the closed zone could create confusion. Thus, this event should be clarified. **Methods:** Electropherograms with minipeak at zone 2 or between zone 2 and zone 3 of 103 subjects, who attended to diagnose thalassemia at the Centre of Research and Development of Medical Diagnostic Laboratories, Khon Kaen, Thailand, were enrolled in this study. All subjects were analyzed for Hb CS and Hb PS genes by ASPCR because Hb CS and Hb PS are observed at the same zone. **Results:** To clarify the suspected electropherograms containing Hb CS or Hb PS, electropherogram and DNA detection of each subject were analyzed. Samples with Hb CS gene, Hb PS gene and none of both genes were 94.17%, 3.88% and 1.94%, respectively. Electropherogram patterns of Hb CS or Hb PS were found as following; one minipeak at zone 2 with or without the amount of hemoglobin (63.1%), one minipeak between zone 2 and zone 3 (31.1%), two of minipeaks at zone 2 and at zone 1 (2.9%), and two of minipeaks between zone 2 and zone 3 and at zone 1 (2.9%). Furthermore, the amount of Hb CS or Hb PS in the presence of single gene of Hb CS or Hb PS was 0.1%-1.3% for Hb CS or Hb PS trait, and 0.4%-4.1% for Hb H disease whereas that of two Hb CS genes was 3.4%-5.1%. **Conclusions:** The results indicate that the most common of minipeak at zone 2 or between zone 2 and zone 3 on electrophrogram of capillary zone electrophoresis is Hb CS and the less common of that is Hb PS. This information is useful for reporting Hb CS in the studied area.

734 SIX-DAY STABILITY OF RETICULOCYTE ANDERYTHROCYTE PARAMETERS IN-VITRO: A COMPARISON OF BLOOD SAMPLES FROM HEALTHY, IRON DEFICIENT AND THALASSEMIC INDIVIDUALS

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**Introduction:** Six-day stability data of reticulocyte and erythrocyte parameters in blood samples from patients with iron deficiency and thalassemia has not been reported. This knowledge deficit challenges hemoglobinopathy work-up of referral samples with advanced sample age. We aimed at establishing such data. **Methods:** Our routine hemoglobinopathy work-up includes full blood count (Sysmex XE-2100), ferritin and C-reactive protein (CRP) measurement (Cobas 8000, Roche), chromatographic hemoglobin (Hb) phenotyping (Variant, Bio-Rad), and gene analysis for the seven most common deletional alpha-thalassemias. After a confirmed diagnosis, we included eight samples from healthy (normal concentrations of Hb and ferritin), eight samples from patients with iron deficiency (microcytosis and ferritin concentration ≤10 μg/L) and eleven samples from patients with thalassemia (microcytosis and ferritin concentration ≥20 μg/L) stored at room temperature and with a sample age less than four hours. Subsequently stored at room temperature, analysis of reticulocyte and erythrocyte parameters was performed every 24 hours for six days and percentage change from baseline was calculated. Statistical significance was defined as p<0.05. **Results:** We found that Hb concentration, mean corpuscular hemoglobin (MCH) and number of erythrocytes (RBC) were stable in all samples throughout the study period. Mean corpuscular volume (MCV) increased as expected upon storage, but significantly less (p<0.05) in samples from patients with iron deficiency, possibly due to lower mean corpuscular hemoglobin concentration (MCHC) and thereby a smaller osmosis effect as compared to healthy. Number of reticulocytes increased in all samples from 48-hour sample age, probably due to a method unspecificity. The increase was larger (p<0.05) in samples from patients with thalassemia than in samples from healthy. Surprisingly, we found reticulocyte hemoglobin equivalent (Ret-He) stability to depend on its baseline level. Ret-He increased more in samples with a very low Ret-He baseline value than in samples with a somewhat higher baseline value (p<0.05). **Conclusions:** Hb, MCH and RBC are stable in-vitro and can thus be trusted in samples with advanced sample age in hemoglobinopathy work-up. Ret-He stability seems to depend on baseline level.
736 ERYTHROCYTE SUBSETS IN THE DIFFERENTIAL DIAGNOSIS OF MICROCYTIC ANEMIA
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Introduction: Thalassemia and iron deficiency are the most frequent causes of microcytic anemia. Various indices derived from red blood cell (RBC) parameters measured with automated hematology analyzers have been described for distinguishing these diseases. Such indices were proven to be efficient selection tools of samples that need further testing for confirming the diagnosis. Applying Mie theory principles, the CELL-DYN Sapphire analyzer (Abbott Diagnostics, Santa Clara, California, USA) uses multi-angle laser light scattering for measuring various RBC subsets, including microcytic (M) and hypochromic (H) cells. We studied the M/H ratio as a discriminant index in microcytic anemia and compared it to more traditional indices. Methods: During a period of 4 months (April to July 2013) we selected 261 patients with microcytic anemia (Hb 7.0 g/dL, MCV < 80 fL) from the routine workload. Standard diagnostics confirmed that 160 of them had iron deficiency and 101 were beta thalassemia carriers. Samples were analyzed on a CELL-DYN Sapphire within 6 hours from blood collection and discriminant indices calculated. Optimal cut-off values were established using ROC analysis. These values were used in the validation set of 267 patients (123 with Iron deficiency, 77 beta thalassemia carriers, 67 with other microcytic anemia), collected similarly in the period September 2013 to December 2013. Results: A M/H ratio > 6.4 was found to be strongly indicative of beta thalassemia: AUC 0.948, with sensitivity 0.964 and specificity 0.851. The traditional England-Fraser, Green-King and Mentzer indices all had comparable AUC (0.938-0.958), whereas sensitivity ranged 0.666-0.857 and specificity 0.942-0.984. Applying this M/H ratio cutoff value to the validation group resulted in 93.3 % correct classifications of the iron deficiency patients and 97.4 % of the beta carriers. Conclusions: Overall, the M/H ratio performed as well as the Green-King index in identifying thalassemia carriers among patients with mild microcytic anemia. Whereas the Green-King index was more specific, the M/H ratio was the most sensitive, which makes it a quick and inexpensive tool for screening those patients who need confirmatory HbA2 testing.

738 EXPEDIENCE OF THE GREEN AND KING RULE IMPLEMENTED IN THE HEMATOLOGY-ANALYSER SOFTWARE FOR SCREENING FOR HB-PATHIES
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MDC-Amstelland Amstelveen, Netherlands

Introduction: As seen worldwide, dutch healthcare is also under financial strain. Patients are confronted with higher costs in deductibles and more and more laboratory tests fall in this category. The diagnostic repertoire for detecting Hb-pathies are amongst the tests that can rapidly consume the legal limit of 350-euro in deductibles. Therefore, we investigated the expediency of the “Green and King rule” to screen for Hb-pathies, especially thalassemia. Based on the results of complete blood count (CBC), it should be possible to distinguish between those at risk or not. Those at risk should then be evaluated by Hb-electrophoresis and/or molecular techniques for the presence of Hb-variants and/or thalassemia. Methods: We implemented the Green and King rule in the hematology analyzer and evaluated every positive hit (n = 94) that we acquired from November 21st 2013 to February 9th 2014. This was our validation cohort. Results: Of these patients submitted for CBC, 14 out of 94 were known with a Hb-pathy, 80 were unknown. We contacted the physician and conveyed whether they wanted further evaluation for Hb-pathies. Of these patients 13 weren’t submitted to further testing due to elderly age, 23 came back with confirmed Hb-pathy. Forty-four patients are still in the process of evaluation with confirmation tests. Conclusions: The Green and King rule seems a good screening tool for Hb-pathies. Thus far, we yielded no false positive results, although the sample size is relatively small. However, we were also confronted with positive hits in elderly patients. These patients were not evaluated and could comprise false positive results. Therefore, the Green and King rule can be more refined by adding in an age limit around above 65 years of age.

740 ANALYSIS OF RETICULOCYTE PARAMETERS IN HEMOGLOBIN S TRAIT AND HEMOGLOBIN C TRAIT
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Introduction: Analysis of reticulocyte parameters has proved to be useful in the diagnosis of several red cell disorders (anemia of chronic diseases, iron deficiency anemia). However, their role in diagnosis of structural haemoglobinopathies has hardly been assessed. Hemoglobin S trait (HbS) and hemoglobin C trait (HbC) are the most common structural haemoglobinopathies in our geographic area. Methods: All prospectively diagnosed HbS and HbC cases (based on alkaline and acid electrophoretic pattern) in our laboratory between October 2012 and April 2013 were evaluated. The following parameters provided by Advia 2120i analyzer (Siemens Medical Solutions Diagnostics®) were evaluated: Absolute reticulocyte count (Retic), percentage of reticulocytes (%Retic), concentration of reticulocyte Hb (CHR) and reticulocyte subpopulations based on their fluorescence according to mRNA content [low (%L-RTC), medium (%M-RTC) and high (%H-RTC)]. Immature reticulocyte percentage (%IR) = %M-RTC + %H-RTC. IR= M-RTC + H-RTC. Independent sample t-test was used to compare reticulocyte parameters between HbS, HbC and healthy controls, and receiver operating characteristic (ROC) curves were plotted.

Results: Patients: 164 variant Hb (124 HbS and 40 HbC) and 128 normal controls. Significant differences were found between normal controls and variant Hb (HbS + HbC) in: Retic (69.63 vs 75.99, p<0.029), CHR (32.43 vs 29.78, p<0.001), %L-RTC (85.97 vs 79.13, p<0.001), %M-RTC + %H-RTC (72 vs 95.35, p<0.001), %H-RTC (2.01 vs 6.39, p<0.001), H-RTC (11.59 vs 37.42, p<0.001), %IR (14.4 vs 20.87, p<0.001), IR (85.59 vs 132.77, p<0.001). %H-RTC was the most efficient to distinguish HbC from HbS (AUC=0.818), and the best cut-off was 14.5 (SE=74.1%, SP=74.2%). Significant
Hb F >2% (17.09%) and δβ-TT (18.79%) were statistically significant (p<0.001) (Figure 1). RDW showed high discriminant efficiency (AUC = 0.918) and the cut-off of 17.35% provided sensitivity of 84.5% and specificity of 85.9%. Pearson coefficient showed good correlation between the percentage of Hb F and RDW (r = 0.762, p<0.001).

**Conclusions:** The percentage of Hb F determines the degree of anisocytosis in patients with β-TT and δβ-TT. The elevated RDW is probably due to a heterogeneous increase in the expression of gamma globin genes and, consequently, of Hb F. If a patient with laboratory features suggestive of thalassaemia shows elevated RDW as well, not only δβ-TT should be suspected but also β-TT with high levels of Hb F. The best cut-off for RDW to discriminate between β-TT (regardless of the percentage of Hb F) and δβ-TT was 17.35%.

**472 FETAL HEMOGLOBIN DETERMINES THE DEGREE OF ANISOCYTOSIS IN BETA AND DELTA BETA THALASSAEMIA TRAIT**

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**Introduction:** Classical red cell parameters are similar in Beta thalassaemia trait (β-TT) and Delta Beta thalassaemia trait (δβ-TT), with the exception of Red Cell Distribution Width (RDW) which is significantly higher in δβ-TT patients. Heterogeneous distribution of haemoglobin (Hb) F among red cells may lead to two different red cell populations and, consequently, to an elevated RDW in this subjects. However, no statistical correlation between Hb F and RDW has been reported to date.

**Methods:** Three groups of patients were prospectively studied: 97 δβ-TT (Hb F >3% and Hb A2 < 3.4%), 56 β-TT (Hb A2 >3.4% with Hb F >2% and 21β-δ-TT (Hb A2 >3.4%) con Hb F <2%. In doubtful cases, sequencing of the beta globin gene was performed. Complete blood count was performed on Advia 2120i analyzer (Siemens Medical Solutions Diagnostics, Tarrytown, NY, USA) in all of them. Hb A2 and Hb F levels were determined by high-performance liquid chromatography (HPLC) in the HA-8160 analyzer (Menarini Diagnostics, Florence, Italy). Ferritin, transferrin and transferrin saturation index (TSI) were measured by chemiluminescence immunoassay in the Advia Centaur (Siemens). Patients with thalassaemia and concomitant iron deficiency (ferritin <20 ng/ml and/or TSI <20%) were excluded. Pearson coefficient was estimated to assess the correlation between Hb F and RDW and ANOVA-test performed to compare RDW between β-TT and δβ-TT. Receiver operating characteristic (ROC) curve was plotted to select the best cut-off of RDW. The statistical software package SPSS was used for statistical analysis of the results.

**Results:** Differences in RDW values in β-TT with Hb F <2% (15.74%), β-TT with Hb F >2% (17.09%) and δβ-TT (18.79%) were statistically significant.

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Introduction: Systemic mastocytosis (SM) is a rare myeloproliferative disorder characterized by an accumulation of neoplastic mast cells (MC) in one or more extracutaneous organs. The 2008 World Health Organisation (WHO) diagnosis of SM includes one major criterion (multifocal infiltrates of ≥15 MC in bone marrow (BM) and/or extracutaneous organs) and four major mutation; and 4) expression of CD2 and/or CD25. Results: Conclusions: Despite the normal PK/HK ratio in the patient, the finding of a slightly increased value in both parents (father: 15.8 and mother 15.4; RV: 7.5-15.5) lead us to the suspicion that they may be heterozygote carriers of HK deficiency. In clinical practice, PK/HK ratio is a useful indicator of PK deficiency when this enzyme defect is jeopardized by the increased number of young RBC populations. Accordingly, in PK deficiency associated with high reticulocytosis, the PK/HK ratio is in general lower than normal (7.5 to 15.5) because HK activity, another age dependent RBC enzyme, is two or three fold higher than PK activity.

Results: Conclusions: Despite the normal PK/HK ratio in the patient, the finding of a slightly increased value in both parents (father: 15.8 and mother 15.4; RV: 7.5-15.5) lead us to the suspicion that they may be heterozygote carriers of HK deficiency. In clinical practice, PK/HK ratio is a useful indicator of PK deficiency when this enzyme defect is jeopardized by the increased number of young RBC populations. Accordingly, in PK deficiency associated with high reticulocytosis, the PK/HK ratio is in general lower than normal (7.5 to 15.5) because HK activity, another age dependent RBC enzyme, is two or three fold higher than PK activity.

Results: Introduction: Background: Patient safety and well-being mandates that medical laboratories maintain accuracy and reduce errors in all phases of specimen analysis. However, despite all efforts, the most testing errors occur in the pre-analytical stage, with specimen identification and labelling being the biggest culprits. Incorrect identification of a blood sample can result in incorrect treatment, wrong blood transfusion and may even lead to the patient’s death. Aim: The objective of this study was to determine the incidence of non-conforming blood samples in the general haematology laboratory, at the Sultan Qaboos University Hospital from 2007 to 2010 and by taking appropriate corrective action, evaluate the efficiency of the preventive measures that were implemented. Methods: Method: Data of the specimen records for the year 2007 to 2010 were collected and analyzed. Non conforming samples were defined using the following categories: clotted, haemolyzed, wrong anticoagulant, quantity not sufficient, wrong or no label and no sample received. Based on the available records for the year 2007, corrective actions were initiated in the year 2008, including several educational training activities like proper labeling, collection and handling of blood samples. Results: Results: A total of 63,800 laboratory specimens were received in 2007 with 184 (0.29%) non conforming specimen. After starting the corrective measures in 2008, we found that the total specimen received with the percentage of non conforming samples were respectively 81,024 (0.29%), 93,763 (0.25%) and 1,02,400 (0.21%) for the years 2008, 2009 and 2010. Conclusions: Summary/ Conclusion: Despite the increase in the specimen numbers through the years up to 2010 by 60.5%, the study showed a clear reduction in the non conforming specimen from 2007 to 2010 by 0.08%, indicating a 28% improvement. Specimen identification errors showed a reduction of 0.051% with 87.9% improvement. The study demonstrates the value of the educational activities taken by the haematology laboratory staff and emphasizes the need for providing attention to specific categories of the non conforming specimen samples to get better results.
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LABORATORY REPORTING OF PERIPHERAL BLOOD COUNTS IN ITALY AND EVIDENCE BASED MEDICINE
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Introduction: The GdS -E SIMeL 2012-13 survey is intended to point out the reporting characteristics in Italian hematology laboratories. Methods: 148 colleagues replied to a 24-item questionnaire including questions on the characteristics of reporting and communication to end users (clinicians, GP and outpatients). Results: 69% of the sample encoded reporting criteria and 62.1% declared to apply GdS-E SIMeL guidelines (2002). 85.4% do not provide raw data from instruments (eg dot plots, histograms) to the clinicians/GP. As to parameters provided, the report can include RDW (78.3%), MPV (48%) and IFR (13.5%). Comments are present in 85.8% (standard, encoded, standardized and shared). 76.8% use standardized comments, 24.3% encoded: among them, 73.6% declare a proactive behavior in diagnostics, 42.2% suggesting further, more specific tests, 16.5% diagnostic chances and 41.3% suggesting both solutions. 27.7% use automatic laboratory flow-charts, mainly for flow-cytometry cell typing (29.6%), or for the etiologic agent demonstration in suspected infections (24.4%). Second diagnostic level in studying anemia (21.9%), more rarely coagulation and monoclonal protein diagnostics (cumulative 7.3%) are also set up. Conclusions: About two thirds of the professionals follow guidelines (GL); the number of laboratories which don’t know or apply GL is higher in smaller hospitals and in the centre-south Italy. Some laboratories still provide raw data from instruments and/or parameters not standardized. The use of comments is widespread but rarely shared. All positive percentages are higher in North-Eastern Italy and in midsized hospitals. Proactive flowcharts are implemented by less than 30% of professionals and again in North-Eastern Italy and in midsized hospitals. The latter, with case-mix requiring expertise, appropriate test number and adequate working-experience team, allow hematology laboratory section where specific skills can be developed. Most of the above-mentioned survey reports suggested the opportunity to review previous SIMeL-GdS-E GL about laboratory hematology reporting, also according to the EBLM.

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IS LABORATORY VALIDATION OF PERIPHERAL BLOOD CELL COUNTS IN ITALY EVIDENCE-BASED?
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Introduction: In 2012-13 SIMeL GdS–E promoted a survey about the managing characteristics of the laboratory hematological diagnostic in Italy. The purpose of the survey was to highlight the changes in the management of the hematological laboratory process occurred during the past decade. Methods: 148 colleagues replied to a 24-item questionnaire including questions about validation rules of peripheral blood cell counts (CBC), criteria and impact of the microscopic review, characteristics of report and communication to the clinicians. Results: In the validation/interpretation of CBC, 63% of the laboratories use instrumental erythrocyte Wintrobe indices (58%) and RDW (32%), while WBC technological parameters (such as MPO, LUC, IG, etc.) are considered by about 50% of professionals. Platelet instrumental parameters and/or flags are widely provided (49.3%; MPV: 52%); reticulocyte parameters are also currently used. The smears review represents 5% (mean 5.9%/a day, ranging 1-20) of routine CBC. 83% of the participants in the survey use both clinical and technical selection criteria, 5.5% of them using clinical ones and 6.5% only technical. WBC differential is performed in 60.6% (36.2% in auto-mode and 3.2% in mixed-mode, linked to the CBC numerical amounts). Auto-review occurs by instrumental rules (19.4%), by LIS (19.4%) and/or by middleware-algorithms (61.2%). 77.7% of the review rules are standardized and agreed inside the team, 13.5% are shared and 25.6% coded. Conclusions: The instrumental data about cell characteristics (resulting by direct measurement or verified by different analytical philosophies and technologies) are widely used in the interpretation and validation of CBC. Some data produced by the instruments (eg the well-known case of RDW) can suggest to select microscopic revisions in validation and interpretation guide and can in particular cases activate some specific diagnostic flow-chart; instrumental data are also widely reported. Selection criteria are rarely shared with clinicians. As to the survey results, the team members seem to share review rules and selection criteria, often without scheduled scheme and/or binding correlation behavior. The survey report suggests that promotion and standardization of validation rules related to the hematology diagnostic process is an important and basic aspect for SIMeL-GdS-E scientific and educational activity.

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COMPARISON BETWEEN N-LATEX AND FREELITE ASSAYS FOR MEASURING FREE LIGHT CHAINS IN A CLINICAL HEMATOLOGY LABORATORY.
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Introduction: Serum Free Light chains (FLC) are valuable markers for the diagnosis and monitoring of patients with plasma cell dyscrasia. Despite its analytical limitations, the established polyclonal antibody-based assay (Freelite, The Binding Site, UK) was the first to enable FLC quantification and detection of subtle changes in early response to therapy or relapse. With the presentation of a novel monoclonal antibody-based assay (N-Latex, Siemens, Germany) careful validation studies for comparison between the two assays are essential. The aim of this study was to validate the compatibility between the two assays for use in a routine clinical laboratory. Methods: FLC was quantified in 167 samples taken from 135 patients by both Freelite and N-Latex kits on IMMAGE turbidimeter and Behring BN-Prospec nephelometer, respectively. Serum Immunofixation electrophoresis was performed in samples having greater than 5-fold difference in FLC levels between the two methods. Results: Sixty-seven percent of the samples were obtained from multiple myeloma patients, of which 49% produced intact immunoglobulin clone and the rest had light chain myeloma. Overall, N-Latex FLC values were significantly higher in levels up to 100mg/L (p<0.001) and lower in levels above 100mg/L (p<0.001), compared to Freelite results. Moreover, in the low to normal range of Freelite kit, 7% of kappa and 28% of lambda measurements had elevated-abnormal concentrations according to N-Latex reagent. FLC ratios from both kits were in agreement...
as to monoclonality in 94% of the cases, despite differences in their absolute values. Samples from 24 patients during follow-up period were analyzed, of which 54% had consistent low levels of kappa and lambda (<50 mg/l by both assays). In samples from patients with active disease, the trend of change was similar between methods, although significant differences in the absolute values were observed which probably arose from the distinct immune complexes formed by the polyclonal and monoclonal reagents. Immunofixation was used to evaluate absolute FLC levels in samples from 4 patients having marked differences between assays, 3 of them were in accordance with the Freelite quantitation. Conclusions: N-Latex and Freelite assays are mostly comparable for diagnosis and monitoring, despite the significant differences in their FLC absolute levels. However, further prospective validation in larger cohorts of patients is needed. In addition, N-Latex implementation requires an extended bridging period with Freelite assay, during which disagreement between methods could be resolved by immunofixation. Future efforts should be directed to produce FLC international standard which will be aligned with consensus guideline criteria.

Comparison of Freelite (TLS) and N-Latex (Lambd4) serum free light chain assay on a logarithmic scale. N-Latex FLC values were higher in FLC concentration up to about 300 mg/L for both kappa and lambda, p>0.001) and lower in concentration of more than 500 mg/L compared to Freelite kit (kappa p<0.001, Lambda non-significant trend). (A) Kappa (n=100) (B) Lambda (n=121). Solid line is the line of identity. Dotted line is regression line. Normal range of Siemens (Sysmex) is (Kappa 0.5-22.6 mg/L, Lambda 8.3-27) and TLS (vertical Kappa 3.3-19.4 Lambda 5.7-36.5). Grey area shows detection limit of serum immunofixation (90 mg/L). Dots recorded as less than the detection limit for each assay, could not be incorporated in the correlation analysis and were excluded.

Introduction: The Spanish Society of Hematology and Hemotherapy started a web-based external quality assessment scheme in hemato logical cytogenetics (EQAS-CG) in 2009, in order to provide a tool for self-evaluation in cytogenetic analysis of clinical cases and to promote the adherence to the International System for Human Cytogenetic Nomenclature (ISCN) in karyotype description. Twenty images of each case must be downloaded from the website four times a year. The results are received from 18 surveys. 96.5% of laboratories identified the anomaly in the twelve cases in which only one was present, 88% of them used correct nomenclature and indicated the correct chromosomal breakpoints. 79.4% identified all of the anomalies in the five cases that had more than one aberration; 85.8% expressed the karyotype properly. 92.5% correctly reported the normal karyotype in the only normal case submitted. The most frequent errors in the description of the karyotype were: absence of the sex chromosomes, wrong number of chromosomes, and the order of the anomalies when there was more than one. Conclusions: The software allowed us to detect automatically failures in the accurate identification of chromosomal anomalies and in the description of the karyotype using a correct nomenclature.

810 COMPARISON OF HEMOGLOBIN ON RAPIDPOINT 500 BLOOD GAS ANALYSER (SIEMENS) TO XE-5000 CELL COUNTER (SYSMEX).

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Introduction: Besides the conventional automated cellcounters in the lab, blood gas devices are increasingly used to provide point-of-care testing (POCT) of hemoglobin concentration (Hb). As the Hb measurements are interpreted bedside regardless of which method used, a good agreement with the result on the cell counter is mandatory. This study evaluates whether Hb on RAPIDPoint 500 blood gas system (RP500) is interchangeable with that on XE-5000 automated blood cell counter. Both methods use different measurement principles. Methods: Routinely prefilled heparinized arterial blood gas syringes (ABG) (Monovette 2mL, Sarstedt, Germany) and EDTA vacutainer tubes (K2 EDTA 5.4 mg. 3.0 mL, BD, USA) from patients admitted to the ICU were collected. These tubes were prefilled simultaneously through the same arterial catheter. Hb on ABG was tested randomly on maximum five different RP500s (Siemens, Germany) and EDTA analysis on two XE-5000 analyzers (Sysmex, Japan). Statistical analyses were performed using MedCalc (MedCalc, Belgium). Results: During 18 days, 75 ABG and EDTA tubes were collected from 33 different ICU patients. Hb on RP500 ranged from 6.4 to 14.3 g/dL (mean 10.5 g/dL) and on XE-5000 from 5.9 to 13.3 g/dL (mean 9.7 g/dL). The overall correlation between Hb on XE-5000 (x) and RP500 (y) was y=0.832+0.104x with the correlation coefficient r=0.8792 (95%CI [0.9714 to 0.9849]). Using the method of Bland and Altman, the overall mean difference of Hb between XE-5000 and RP500 was -0.54 g/dL (8.4%) (95%CI [-0.8946 g/dL to -0.1908 g/dL].
the mean differences among the RP500 analysers ranged from 0.02 g/dL to 0.16 g/dL. **Conclusions:** Both methods correlate well but RP500 overestimates Hb on 5 different analysers. Much smaller biases were found among the RP500s. Results from both measurements are not interchangeable because the bias is higher than the within-subject biological variation of Hb. Hb measured on XE-5000 is considered correct as it is controlled by external quality programs. To make both measurements interchangeable, correction factors (intercept and regression coefficient) at the level of the RP500 software are established. This study shows that when reporting to clinicians a parameter measured by different assay principles, one should perform a correlation study.

**812 LONGITUDINAL EVALUATION OF HEMATOLOGICAL VALUES OBTAINED WITH REFERENCE AUTOMATED HEMATOLOGY ANALYZERS OF SIX MANUFACTURERS (2013)**

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**Blood Cell Count Standardization Subcommittee, Japanese Society for Laboratory Hematology Tokyo, Japan, 2Japanese Society for Laboratory Hematology Tokyo, Japan**

**Introduction:** To promote the standardization of hematological values, we did the longitudinal evaluation by holding the fresh-blood-based survey for the reference automated hematology analyzers of 6 manufacturers in 2001, 2005, 2007, 2010 and 2013. **Methods:** Blood samples – 65 ml of fresh whole blood anti-coagulated with EDTA-2K was collected from 3 healthy volunteers. Aliquot volume (3.5 ml) of whole blood samples were distributed to each laboratory for analysis of complete blood count (CBC), leukocyte differential counts and reticulocyte counts. **Measurement –** The survey samples were measured (5-run per each sample) within 4 hours after drawing, on the representative reference hematology analyzers at the six manufacturers’ laboratories – DxH800 (Beckman-Coulter), XN-2000 (Sysmex), ADVIA2120 (Siemens), MEK-7300 (Nihon Kohden), PENTRA60 (Horiba) and CELL-DYN Sapphire (Abbott). **Evaluation –** Comparison of 6 manufacturers’ results: historical changes of the ratio of each manufacturer’s data to the mean values for each sample were evaluated. **Results:** From this study, we found the following points; 1. For HGB, MCV and WBC, wider variation was observed among the 6 manufacturers’ data in the 2001 survey than previous surveys. 2. For RBC and Hct, stable variation (+/-3%) was observed among the 6 manufacturers’ data through five surveys. 3. For Plt, wider variation (+/-10%) still was observed since the 2001 survey. 4. For leukocyte differential, good agreement was observed for neutrophils and lymphocytes, but wider variation was observed for monocytes, eosinophils and basophils. 5. For reticulocyte counts, a significant difference was still observed among the 5 manufacturers’ data since the 2001 survey. **Conclusions:** Increasing trends of difference among hematology analyzers of different manufacturers were observed in several items of hematology values. In these items, a similar trend will also be present in the results of routine clinical examination. For the manufacturers of hematology analyzer, this study provides the opportunity to know the secular trend of the measurements. We could utilize these findings effectively in order to correct for the differences among hematology analyzers of different manufacturers.
THE FIRST STEP TO ESTABLISH EXTERNAL QUALITY ASSESSMENT SCHEME FOR POINT-OF-CARE INR IN THAILAND
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Introduction: The effective warfarin therapy management has gained national interest, and results in the increased use of point-of-care (POC) testing for international normalized ratio (INR). The external quality assessment (EQA) is recommended to ensure quality of the testing. CoaguChek XS and CoaguChek XS plus are the only commonly available POC-INR monitors in Thailand. Therefore, Thailand NEQAS for Blood Coagulation initiated the scheme for POC INR in 2013, including only hospitals using these devices. Methods: Questionnaires were sent to the hospitals, inquiring about their interest in participation of EQA program and the details concerning POC-INR testing. Two surveys were distributed at the interval of six months. Each survey contained five certified European Concerted Action on Anticoagulation (ECAA) INR plasma sets. Unsatisfactory performance was indicted by a 15% deviation from the certified mean INR value. Results: There were 156 hospitals using the devices. Thirty-five hospitals responded the questionnaires. Medical personnel undertaking POC-INR test were confined to laboratory staff in 29 (83%) of these centers. However, 31 hospitals actually participated in the EQA scheme. Results of the two surveys are shown in table 1. The same batch of plasmas was used in both surveys. The certified mean and the overall median of INRs were similar. No systematic bias or persistent unsatisfactory performance was observed. Table 1. The certified mean INR and acceptable range of ECAA plasma sets and the results of point-of-care INR in survey 1 (N = 32, there was one lab sending results of three monitors) and survey 2 (N = 29). (The bold and italic numbers are three results outside acceptable range from three laboratories)

<table>
<thead>
<tr>
<th></th>
<th>Sample 1</th>
<th>Sample 2</th>
<th>Sample 3</th>
<th>Sample 4</th>
<th>Sample 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Certified mean INR (acceptable range)</td>
<td>2.2 (1.9-2.6)</td>
<td>2.7 (2.3-3.2)</td>
<td>1.9 (1.6-2.3)</td>
<td>3.5 (2.9-4.1)</td>
<td>2.4 (2.0-2.8)</td>
</tr>
<tr>
<td>Median INR (range) of survey 1</td>
<td>2.2 (2.2-2.5)</td>
<td>2.7 (2.2-3.3)</td>
<td>1.9 (1.9-2.1)</td>
<td>3.2 (3.1-3.3)</td>
<td>2.4 (2.3-2.6)</td>
</tr>
<tr>
<td>Median INR (range) of survey 2</td>
<td>2.2 (2.1-2.4)</td>
<td>2.6 (2.5-3.1)</td>
<td>1.9 (1.8-2.1)</td>
<td>3.3 (3.1-3.4)</td>
<td>2.4 (2.2-3.3)</td>
</tr>
<tr>
<td>CV (%) of survey 1</td>
<td>3.0</td>
<td>6.1</td>
<td>2.9</td>
<td>2.2</td>
<td>3.1</td>
</tr>
<tr>
<td>CV (%) of survey 2</td>
<td>3.5</td>
<td>3.9</td>
<td>3.8</td>
<td>2.3</td>
<td>7.9 (2.5)*</td>
</tr>
</tbody>
</table>

* Excluding one outlying result which is INR 3.3

Conclusions: The EQA for POC-INR in Thailand is feasible, though limited to one type of monitor. Performance evaluation based on certified acceptable range is comparable to the allowable deviation from the overall median INR used in the conventional EQA analysis.

POSTER SESSION 2

101 VCS PARAMETERS LIKE MNV, MMV AS EARLY MARKERS OF SEPSIS AND TREATMENT RESPONSE
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Introduction: Sepsis is a major cause of morbidity and mortality in hospitalized patients worldwide which can be reduced by early diagnosis and prompt initiation of therapy. The condition is associated with morphologic changes in the size and granularity of leukocytes. This can be measured using the volume, conductivity and scatter (VCS parameters) obtained from the automated hematology analysers. These parameters can serve as better and rapid markers for the prediction of sepsis. The objective of this study is to find out the clinical usefulness of VCS parameters as possible indicators of sepsis and to determine the effect of treatment on these parameters. Methods: This observational study was conducted in the Department of Laboratory Medicine, All India Institute of Medical Sciences, New Delhi between April 2013 to December 2013. Hemogram along with VCS parameters obtained from LH750 (Beckman coulter, Fuellerton, CA) from 134 blood culture positive cases received on day of diagnosis (day 0) were retrospectively reviewed. Out of these VCS parameters of 110 patients were reviewed again on day 3 and day 7 after initiation of treatment. Samples from 40 healthy subjects were taken as control and their VCS parameters were compared with that of the cases. Statistical analysis of data was done and cut-off value was established using ROC curve.

Results: The mean age and gender ratio of the study group was comparable to the controls (32± 10 vs 33± 8), p=0.7 for age and (1.06 vs 0.9), p=0.65 for M:F ratio. Out of 134 culture positive cases, in 55%(n=74) gram negative and in 45%(n=60) gram positive bacteria were isolated. The most common organism isolated was Staphylococcus aureus 32%(n=43) followed by Escherichia coli 17.2%(n=23). The mean neutrophil volume (MNV) and mean monocyte volume (MMV) were higher in the sepsis group compared to control group (165.4± 18.21 vs 140.59± 7.6, p=0.001 for MNV and 179.8± 14.16 vs 164.54± 9.6, p=0.001 for MMV). A significant decrease in MNV and MMV was observed with the initiation of treatment. A cut-off value of 150.1 for MNV gave a sensitivity and specificity of 79.85% and 95% respectively with an AUC of 0.92. With a cut-off of 168.7 MMV had a sensitivity of 80.6% and specificity of 77.5%, AUC of 0.82.

Conclusions: VCS parameters like MNV and MMV can be easily obtained by an automated hematology analysers and can be used for early detection and therapeutic response in sepsis.

103 AUTOMATED SCREENING FOR MALARIA PARASITES ON MINDRAY BC-6800 HEMATOLOGY ANALYZER: A FOLLOW-UP REPORT
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Introduction: Malaria is a globally spread vector borne disease causing high morbidity and mortality. Careful microscopic review of thick blood film to detect malaria parasites is considered the Golden reference method. While the method is known to be
subjective, labor intensive requiring high expertise; the alternative Immuno-chromatographic rapid diagnostic tests (RDT) though more reliable, are seen as expensive as mass screening tool.

Methods: 948 patients with symptoms suggestive of malaria infection and referred to the Lab to rule out or confirm the possibility; were included in the present study. All samples were tested by (A) the reference method viz. microscopy examination of thick blood films to rule out presence of at least one of the three forms of malaria parasites i.e. trophozoites, schizonts and/ or gametocytes of Plasmodium vivax or falciparum and (B) RDT. All blood samples were also tested on BC-6800 analyzer and “infected RBC?” flag was evaluated for its sensitivity, specificity & predictive value in malaria screening. Results: Malaria parasite presence was confirmed in 52 of 948 patient samples by reference method. Of these, 39 had P. vivax infection while 13 had P. falciparum parasites. Remaining 896 blood samples did not show presence of malaria parasites by reference method. Since the cases with P. falciparum infection were only 13, these were not analyzed statistically as a separate group. Considering P. vivax malaria cases alone (n=39), for the “infected RBC?” flag showed 92.3% Sensitivity, 100% Specificity and 99.7% Negative Predictive value (NPV). When both forms of malaria were considered (n=52) “infected RBC?” flag’s overall Sensitivity was 78.85 %, Specificity 100% & NPV 98.79 %. Conclusions: The “infected RBC?” flag on Mindray BC-6800 showed excellent sensitivity, specificity and NPV in detecting P. vivax parasites. We have reconfirmed diagnostic utility of “infected RBC?” flag (available as standard on the analyzer) as an efficient tool to screen for malaria parasites, even in unsuspected cases. Since this, our parameters have a good predictive value to rule out possible presence of malaria parasites and therefore could prove be very useful in malaria endemic area.

105 BLOOD CELLS ANALYSIS IN CEREBROSPINAL FLUID USING SYSMEX® XN-1000 HEMATOLOGY ANALYSER

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1University Medical Centre Ljubljana Ljubljana, Slovenia, 2Community Health Centre Hrastnik, Slovenia

Introduction: Cell count in cerebrospinal fluid (CSF) is one of the basic but important routine laboratory parameters to diagnose several neuroinflammatory diseases. In most laboratories, the microscopic reference method is applied for cell count using a Fuchs-Rosenthal counting chamber. The method is time and labour consuming and requires experienced laboratory staff; however, it is still associated with intra- and interoperator variability. The hematology analyzer Sysmex® XN-1000 with improved body fluid (BF) mode for counting white blood cells (WBCs), red blood cells (RBCs) and two-part WBC differential was evaluated and compared to the manual microscopic count. Methods: From April to June 2013, the Institute of Clinical Chemistry and Biochemistry (University Medical centre in Ljubljana) received 107 CSF samples for routine CSF analysis. They were included in the evaluation. Manual microscopic cell counting was performed in a Fuchs-Rosenthal counting chamber after staining WBCs with Samson reagent. We also differentiated cells into polymorphonuclear (PMN) and mononuclear (MN) WBCs. CSF samples were analyzed using XN-1000 in the open mode. The analysis required 88 µL CSF. The analyzer used a polymeathine dye-binding nucleic acids and semiconductor laser technology with Fluorescence Flow Cytometry. Low and high BF XN control samples were measured on a daily basis prior to the analysis of samples. Statistical analysis was carried out with Analyze-it software, version 2.30. Comparisons were performed by using a Bland-Altman test and Pearson’s correlation. Results: A total of 107 samples were included. The WBC concentration ranging from 0 x 10^6/L to 500 x 10^6/L. In the majority of samples, RBC concentration was below 1000 x 10^6/L and not included in the statistical calculation. Between-run precision was assessed and the coefficient of variation equalled 5.5% for WBC (n=22), 2.7% for RBC (n=22), 5.8% for MN (n=22), and 7.0% for PMN (n=22). Bland-Altman analysis showed slightly lower cell count for WBC (bias: -0.7, 95% confidence interval (CI): -3.8–2.5), and PMN (bias: -1.1, 95% CI: 3.0–0.8) and higher count for MN (bias: 1.5, 95% CI: 1.3–4.4) with the XN-1000. The comparison of the manual and automated methods yielded correlation coefficients (R^2) ranging from 0.92 to 0.97. Conclusions: Small biases and a good correlation between the manual and automated methods indicate that the BF module using the XN-1000 is appropriate for cell counts in CSF. The method has advantages because it is fast, standardised and fully automated.

107 INTEREST OF THE DXH-800 HEMATOLOGICAL ANALYSER FLAGS ASSOCIATED WITH CYTODIFF® TO DETECT CLINICALLY RELEVANT SUBCLINICAL B CELL MONOCLONALITY.

Jonathan Brauner, Nicolas Istantes, Hazar Lahlou, Zaina Kassengera, Ingrid Beukinga, Olivier Pradier
ULB-Hôpital Erasme Brussels, Belgium

Introduction: Automation is the future challenge for hematology laboratories. Hematoflow is a workflow based immunodifferential solution that has been introduced in order to change the current leukocyte differential paradigm and improve both accuracy and precision. The initial screening tool to investigate alarms for abnormal cells is based on flow cytometry, providing four new populations: blasts, immature granulocytes, B lymphocytes and T/NK lymphocytes. Hematoflow allows to detect Monoclonal B lymphocytes even with normal total lymphocytes count. We report our experience of 3 years use of Hematoflow in daily laboratory practice. Methods: Hematoflow® system (Beckman Coulter) includes two automatic blood cells analysers (DxH8000) connected to the cytometry platform (sample preparator (FP1000) and flow cytometer (FC500)). The cytometry reagent, Cytodiff®, contains a specific antibody for B lymphocyte (CD19-ECOD). We included patients with a “variant lymph” flag, normal lymphocytes count (total lymphocytes <4.0 x 10^9/L), B cells equal or greater than 0.52 x 10^9/L (mean + 3 standard deviations) and T/NK cells lower than 3.2 x 10^9/L (mean + 2 standard deviations). Samples were assigned to flow cytometry immunoglobulin investigation for kappa/lambda light chains clonality, except in case of inflammatory syndrome. A kappa/lambda ratio >2.5 or <0.7 induced the execution of a complete lymphoma flow immunophenotype investigation. Results: During the study’s period, we performed 403103 WBC differential counts and 34461 CytoDiff. 318 patients were included. 228 patients had polyclonal B lymphocytosis. We identified 90 cases of new B-cell clonality or relapse of a previously known lymphoma. Adaptation of the medical treatment occurred for 25.6% of these cases. Among B-cell clonality, we found essentially monoclonal B cell lymphocytosis (MIB) with a chronic lymphoid leukaemia (CCL) phenotype (N=42) or marginal zone lymphoma (N=30). Other lymphomas included follicular lymphoma (N=3), splenic marginal zone lymphoma (N=2), hairy cell leukemia (N=2), mantle cell lymphoma (N=3), lymphoplasmacytic lymphoma (N=3), diffuse large B-cell lymphoma (N=2) and non-Hodgkin lymphoma, not classified (N=3). Conclusions: Hematoflow can
detect a clinically relevant B cell pathology even if the lymphocyte count is normal. From this point of view, HematoFlow appears to improve significantly the identification and the clinical management of patients with a monoclonal B lymphocytosis or circulating lymphomacells.

109 EVALUATION OF RETICULOCYTE HAEMOGLOBIN CONTENT AS MARKER OF IRON DEFICIENCY IN MOROCCAN HAEMODIALYSIS PATIENTS
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Introduction: Renal anemia is a frequent complication of end-stage renal failure. Correction of this anemia includes the administration of both recombinant human erythropoietin and intravenous iron, the evaluation of iron status in these patients provides information essential to the planning of adequate treatment, the percentage of erythrocytes with a low hemoglobin content (% Hypo-He) and equivalent of the reticulocyte hemoglobin content (Ret-He), were proposed as alternative to traditional biochemical tests. Ret-He is a new parameter measured during reticulocyte analysis by automated hematology analyzers on the basis of automated fluorescent flow cytometry these values adequate with reticulocyte hemoglobin content. Our aim was to verify the clinical usefulness of biochemical and cellular parameters as predictors of iron deficiency in patients undergoing long term hemodialysis.

Methods: We initially compared Ret-He and Hypo-He with such habitual iron parameters as serum ferritin levels, transferring saturation and serum iron level, secondly we investigated the changement in Ret-He and % Hypo-He during iron supplementation for iron-deficit patients to determine the usefulness of these markers on the monitoring of iron sufficiency. We studied 34 patients undergoing hemodialysis 3 times weekly, and receiving rhEPO therapy. Results: Iron deficiency was defined as having a transferring saturation TSAT<20% or serum ferritin < 250ng/ml, Ret-He mean value was: 30,1 pg, for %Hypo-He it was: 2,22%, a weak correlation was noted between Ret-He and serum ferritin (r: 0,19, p<0,01), like the correlation between Ret-He and TSAT, the coefficient of correlation became higher as serum ferritin level (r: 0,54, p<0,1) with sensibility and specificity: 65,3% and 43,1%, like the correlation between Ret-He and TSAT, the coefficient of correlation became higher as serum ferritin level (r: 0,54, p<0,1) with sensibility and specificity: 65,3% and 43,1%, thirdly we investigated the correlation between Ret-He and ferritin, the coefficient of correlation became lower, (r:0,24, p<0,01), the correlation between %Hypo-He and Ret-He, the correlation was stronger with all of the biochemical markers (r: 0,19, p<0,01), like the correlation between Ret-He and TSAT, the coefficient of correlation became higher as serum ferritin level (r: 0,54, p<0,1) with sensibility and specificity: 65,3% and 43,1%, respectively concerning %Hypo-He, the correlation was stronger with all of the biochemical markers (r: 0,19, p<0,01), like the correlation between Ret-He and TSAT, the coefficient of correlation became higher as serum ferritin level (r: 0,54, p<0,1) with sensibility and specificity: 65,3% and 43,1%, respectively concerning %Hypo-He, the correlation was stronger with all of the biochemical markers (r: 0,19, p<0,01), like the correlation between Ret-He and TSAT, the coefficient of correlation became higher as serum ferritin level (r: 0,54, p<0,1) with sensibility and specificity: 65,3% and 43,1%, respectively concerning %Hypo-He, the correlation was stronger with all of the biochemical markers (r: 0,19, p<0,01), like the correlation between Ret-He and TSAT, the coefficient of correlation became higher as serum ferritin level (r: 0,54, p<0,1) with sensibility and specificity: 65,3% and 43,1%, respectively concerning %Hypo-He, the correlation was stronger with all of the biochemical markers (r: 0,19, p<0,01), like the correlation between Ret-He and TSAT, the coefficient of correlation became higher as serum ferritin level (r: 0,54, p<0,1) with sensibility and specificity: 65,3% and 43,1%, respectively concerning %Hypo-He, the correlation was stronger with all of the biochemical markers (r: 0,19, p<0,01), like the correlation between Ret-He and TSAT, the coefficient of correlation became higher as serum ferritin level (r: 0,54, p<0,1) with sensibility and specificity: 65,3% and 43,1%, respectively concerning %Hypo-He, the correlation was stronger with all of the biochemical markers (r: 0,19, p<0,01), like the correlation between Ret-He and TSAT, the coefficient of correlation became higher as serum ferritin level (r: 0,54, p<0,1) with sensibility and specificity: 65,3% and 43,1%, respectively concerning %Hypo-He, the correlation was stronger with all of the biochemical markers (r: 0,19, p<0,01), like the correlation between Ret-He and TSAT, the coefficient of correlation became higher as serum ferritin level (r: 0,54, p<0,1) with sensibility and specificity: 65,3% and 43,1%, respectively concerning %Hypo-He, the correlation was stronger with all of the biochemical markers (r: 0,19, p<0,01), like the correlation between Ret-He and TSAT, the coefficient of correlation became higher as serum ferritin level (r: 0,54, p<0,1) with sensibility and specificity: 65,3% and 43,1%, respectively concerning %Hypo-He, the correlation was stronger with all of the biochemical markers (r: 0,19, p<0,01), like the correlation between Ret-He and TSAT, the coefficient of correlation became higher as serum ferritin level (r: 0,54, p<0,1) with sensibility and specificity: 65,3% and 43,1%, respectively concerning %Hypo-He, the correlation was stronger with all of the biochemical markers (r: 0,19, p<0,01), like the correlation between Ret-He and TSAT

Conclusions: Ret-He and % Hypo-He seemed to be potentials advantages in the estimation of iron status in hemodialysis patients.

111 PHAGOCYTOSIS OF HEMOZOIN IN VITRO, MONITORED USING HEMATOLOGY ANALYZERS
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Introduction: Hemozoin (Hz), also known as malaria pigment, is the metabolic end product of hemoglobin degradation by Plasmodium parasites. During malaria, Plasmodium parasites digest hemoglobin inside erythrocytes, split off the globin proteins and release heme, which is then oxidized to hemat. Heme-like substances are highly toxic to living cells and Plasmodium parasites have the unique capability of detoxifying it by biocrystallization into chemically inert Hz. When Plasmodium- infected erythrocytes rupture into the circulation, liberated Hz is phagocytized by monocytes and neutrophils. Hz crystals exhibit optical dichroism (birefringence). This means that they are able to change the plane of polarization of incident polarized laser light. This ability has been exploited for detecting malaria infections using polarization microscopy and more recently by automated hematology analyzers of the CELL-DYN family, which are routinely measuring depolarized light scatter for identifying eosinophils. The aim of the present study was to investigate the feasibility of monitoring in vitro Hz phagocytosis in human blood and to explore which blood cells actually ingest Hz.

Methods: Two volunteers donated 10 mL blood, which was anticogulated with heparin. Increasing concentrations (0-100 µM) of a suspension of synthetic Hz, which is identical to natural Hz, in buffered saline were added to aliquots of the blood and incubated in a 37 °C water bath. After 90 minutes and 24 h, the samples were measured on CELL-DYN 3500 and CELL-DYN Sapphire hematology analyzers. The raw data files with list mode data of light scatterers from up to 20,000 white blood cells were analyzed. The analyzer scatterplots were also investigated, in order to find cells with abnormal depolarization as a measure of phagocytized Hz.

Results: In both subjects, depolarizing cells were demonstrable after 90 min incubation. The number of depolarizing cells increased with increasing Hz concentration. After 24 h, there were significantly more depolarizing cells than after 90 min. The CELL-DYN analyzers classified the majority of depolarizing cells as monocytes. A second cluster was classified as eosinophils, but these events were actually neutrophils, depolarizing due to ingested Hz. These findings were confirmed by conventional and polarization microscopy.

Conclusions: We have demonstrated that it is possible to monitor the process of in vitro phagocytosis of hemozoin by blood cells.

113 THE ASSOCIATION BETWEEN HLA-E POLYMORPHISMS AND NK CELL FUNCTION MODULATES HEMATOPOIETIC STEM CELL TRANSPLANT OUTCOME
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1Blood Transfusion Research Center, High Institute for Education and Research in Transfusion Medicine Tehran, Iran, 2Department of Immunology, Monash University Melbourne, Australia

Introduction: HSC is a commonly used treatment for various hematologic malignancies. Following transplantation, NK cells play a curtail role in eliminating tumor cells by recognition of different human leukocyte antigen (HLA)-I molecules including HLA-E. Insight to mechanism by which the HLA-E mediates NK cell activity, this study investigated the relation between HLA-E polymorphism and transplant outcome.

Methods: We analyzed 56 pairs of donors and recipients for HLA-E polymorphism (n=56), CD94/NKG2A expression (n=27) and NK cell cytotoxicity (n=27) during 1st, 2nd, 3rd, 6th, 9th, and 12th month of transplantation. HLA-E...
locus was genotyped by polymerase chain-reaction sequence-specific primer (PCR-SSP) strategy. CD94/NKG2A expression and cytotoxicity were analyzed by flow-cytometry and ³¹Cr-release assay, respectively. Results: HLA-E*0103 homozygous patients significantly showed a better outcome than other genotypes. Follow-up studies of NKG2A expression demonstrated the highest level of receptor in 2nd month of transplant (P< 0.001) followed by a constant reduction starting from 3rd month. The reduction occurred more effectively in patients with HLA-E*0103/0103 (P< 0.001) compared to those of E*0101/0101 (P< 0.01). A converse trend for NK cell cytotoxicity was observed after transplantation, demonstrating higher levels of cytotoxicity for recipients (P<0.001) identified with HLA-E*0103/0103 compared to those who had other genotypes. Conclusions: These data suggest a diverse HLA-E-CD94/NKG2A interaction mediated by ligand polymorphism resulting in a higher level of NK cell functional activity and an improved outcome in recipients with homozygous state for HLA-E*0103 allele compared to those with other genotypes.

115 EVALUATION OF NEW FEATURES ON THE SYSMEX XN-20 HAEMATOLOGY ANALYSER IN A LARGE CHILDREN'S HOSPITAL.

AMANDA JONES¹, Ri Lieners¹, Hitesh Tailor², Carol Briggs²
¹Great Ormond St hospital for Children London, United Kingdom, ²University College London Hospital London, United Kingdom

Introduction: Performance validation of Sysmex XN-20 at our paediatric centre compared XN to Sysmex XE5000 and CLSI differential and tested new features on XN: 1) Low WBC mode (LW): samples WBC <1.0 x 10⁹/L
2) White Cell Precursor Channel (WPC): specifies or removes ‘Blasts/Abn lympho’ flag generated by white cell differential channel (WDF). 3) Fluorescent platelet channel (PLT-F): new RNA dye, increased platelet-specificity. Methods: 300 paediatric K2EDTA samples: 2/3 haematologically abnormal, 1/3 normal. XE5000/XN analysis within 6hrs sample draw. 150 WPC pre-selected plus n=150 WPC reflex. Manual differential (CLSI) all samples. Immunological (reference) platelet count: n=16 XE5000 flag ‘PLT abn dst/ seg’/’fragments’ (XE impedance 1-446 x 10⁹/L)
Results: 1) XN vs XE5000 correlation: \( R² > 0.95 \) (WBC, red cell parameters, platelet impedance and optical, WBC differential) 2) XN LW differential vs CLSI : neutrophils (R²=0.99), lymphocytes (R²=0.94), monocytes (R²=0.95), Eos (R²=0.95) n=24, WBC 0.3 – 1.0 x 10⁹/L. LW WBC v XE5000 WBC: \( R²=0.967 \) n=32, WBC 0.03 – 0.96 x 10⁹/L 3) XN-20 WPC v XE5000: n= 224 (150 WPC pre-selected + 74 WPC reflex)

<table>
<thead>
<tr>
<th>Blasts/Abn lympho</th>
<th>WDF</th>
<th>WPC</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>n=150</td>
<td>n=74</td>
</tr>
<tr>
<td>Abnormality rate</td>
<td>n=51</td>
<td>n=99</td>
</tr>
</tbody>
</table>

Sensitivity (%): 100 100 n/a* n/a 100 100 100 100
Specificity (%): 58.9 65.4 88.2 100 40.8 64.4 53.3 53.3
Overall efficiency (%): 64.7 75.7 54.5 31.8 76.5 56.3
* none true positive

5) Blood films for review: trigger = abnormal WBC flag n=224 XE5000 92 XN-20 WPC 73 6) Platelet count correlation: XE impedance v reference, R² = 0.984, intercept +4.42 XE Optical v reference, R² = 0.992, intercept +11.34 XN PLT-F v reference, R² = 0.997, intercept +0.39 n=11/16 p< 0.001 (XE impedance) Blood film: RBC fragmentation/ anisocytosis/ dimorphism/ WBC pyknosis/ large platelets on 12/16. Conclusions: LW mode: accurate WBC count and differential. WPC ‘blasts’ flag - greater sensitivity than XE5000, specificity and overall efficiency similar. WPC increased specificity and overall flagging efficiency over WDF channel alone, blood film review reduced. WPC reflex testing removed WDF ‘Blasts/ Abn lympho’ flag on 46% samples. Good correlation (R²) for platelet methods against reference, however XE optical channel had much higher intercept value than XN PLT-F on samples with known interferents, potentially affecting clinical decisions regarding platelet transfusion. Work ongoing.

117 EVALUATION OF NOVEL WBC VCS PARAMETERS OBTAINED ON LH750 FOR EARLY DIAGNOSIS OF SEPSIS AND DIFFERENTIATION FROM SIRS.

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Introduction: Sepsis is the body’s systemic response to severe infection. Systemic inflammatory response syndrome (SIRS) is associated with infections and noninfectious insults, like autoimmune disorders, pancreatitis and surgery. Early differentiation of sepsis from SIRS is important to prevent severe sepsis and multi organ failure. CBC, C-reactive protein, procalcitonin and blood culture are used for diagnosis of sepsis but the latter are expensive and time consuming. The LH750 uses VCS technology to quantify morphological characteristics which are reported as numerical values called Cell Population Data (CPD). The CPD parameters of WBCs are mean cell volume, conductivity and scatter along with their respective standard deviations. The aim of our study was to use CPD parameters for early diagnosis of sepsis Methods: Our study consisted of 3 groups. Group1-43 sepsis patients (blood culture +ve &/or PCT>2), Group2-29 SIRS patients and Group3-1528 controls(CBC only). CBC with CPD( Beckman Coulter LH750) ,blood culture and PCT were performed on group 1 and 2.

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Results: ROC analysis for single parameters to-differentiate Sepsis vs.Normal and Sepsis vs.SIRS

<table>
<thead>
<tr>
<th></th>
<th>Sepsis vs normals</th>
<th>AUC</th>
<th>Criterion</th>
<th>Sensitivity</th>
<th>Specificity</th>
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<tr>
<td>NDW</td>
<td>.975</td>
<td>&gt;20.91</td>
<td>95.35</td>
<td>90.04</td>
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<tr>
<td>MNV</td>
<td>.849</td>
<td>&gt;149.25</td>
<td>74.42</td>
<td>88.1</td>
<td></td>
</tr>
<tr>
<td>Sepsis vs SIRS</td>
<td>AUC</td>
<td>&gt;25.517</td>
<td>58.14</td>
<td>96.55</td>
<td></td>
</tr>
<tr>
<td>NDW</td>
<td>0.786</td>
<td>&gt;154.144</td>
<td>58.14</td>
<td>82.76</td>
<td></td>
</tr>
<tr>
<td>MNV</td>
<td>0.734</td>
<td>&gt;154.144</td>
<td>58.14</td>
<td>82.76</td>
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</tr>
</tbody>
</table>

Mean Neutrophil Volume(MNV) of > 149.25 shows a sensitivity of 74.42% and specificity of 88.1% and Neutrophil Distribution Width(NDW) of >20.91 shows a sensitivity of 95.35 and specificity of 90.04% in early diagnosis of sepsis. An NDW >25.52 and MNV > 154.14 can readily distinguish sepsis from SIRS with sensitivity of 58.14 for both parameters and specificity of 96.55 and 82.76 respectively. Multivariate analysis was used which defined a model with 9 parameters - TLC, Neutrophil%, ANC, Eosinophil%, AEC, NDW, Mean Monocyte Volume(MMV), Monocyte Distribution Width(MDW) and Mean Neutrophil Scatter(MNS). Using a cutoff of 0.4109 sepsis can be differentiated from SIRS with sensitivity of 90.48% and specificity of 75.86%, PPV of 84.43% and NPV of 94.6%.

Conclusions: CPD from Beckman Coulter LH and DXH analyzers are capable of early prediction of sepsis and can differentiate sepsis from SIRS at no extra cost. A formula can be incorporated into the LIS to generate flags for sepsis and/or SIRS. This would revolutionize patient care in this group of patients.

119 REFERENCE INTERVAL FOR IMMATURE PLATELET FRACTION IN SYSMEX XN MODULAR SYSTEM
Hanah Kim, Young Jin Ko, Mina Hur, Sang Gyeu Choi, Hee-Won Moon, Yeo-Min Yun
Departments of Laboratory Medicine, Konkuk University School of Medicine Seoul, South Korea

Introduction: Recently introduced hematologic analyzer, the XN modular system (Sysmex, Kobe, Japan), has adopted a fluorescent channel to detect platelets and immature platelet fraction (IPF). This study aimed to establish new reference intervals for %IPF and absolute number of IPF (A-IPF) in the XN system. The data of platelets, %IPF, and A-IPF were also compared between the XN and XE-2100 systems (Sysmex). Methods: Blood samples were obtained from 2,105 healthy adults (1,177 men and 928 women) and were analyzed using both XN and XE-2100 systems. The results of the 2 systems were compared using Bland—Altman plot. The reference intervals for %IPF and A-IPF were defined using non-parametric percentile methods according to the Clinical and Laboratory Standard Institute guideline (C28-A3). Results: The platelet count, %IPF, and A-IPF all exhibited non-parametric distributions. The median age (range) was 43 years (18 - 86 years), and platelet count (range) was 240 x 10^9 cells/L (138 - 432 x 10^9 cells/L). There were no statistically significant differences in %IPF (P = 0.1193) and A-IPF (P = 0.3038) between men and women. The mean difference between the XN and XE-2100 revealed a positive bias in platelets (+8.2 x 10^9 cells/L), %IPF (+1.4%), and A-IPF (+3.3 x 10^9 cells/L). The reference intervals for %IPF and A-IPF in the XN system were 1.0 – 8.2% and 2.50 – 17.50 x 10^9 cells/L, respectively. Conclusions: This large-scale study demonstrates a clear difference of platelet counts and IPF as determined by the XN and XE-2100 systems. The new reference interval for IPF in the XN system, including %IPF and A-IPF, would provide fundamental data for clinical practice and research.
Introduction: A fast and accurate differentiation of white blood cells (WBC) in serous fluids is essential for diagnosis and follow-up of several diseases. Manual microscopic differentiation is however time-consuming, has a high imprecision and inter-observer variability. The aim of this study was to investigate whether automated WBC differentiation on the Sysmex XN 2000 body fluid module could serve as an alternative to the microscopic method. Methods: One hundred and ten samples (35 ascites, 20 pleural fluids, 17 chronic ambulatory peritoneal dialysis and 58 pleural fluids) were analyzed on the body fluid module of Sysmex XN 2000, which offers a 2-part differentiation into mononuclear (MN) and polymorphonuclear (PMN) cells (CE/IVD-labeled) and an extended 4-part WBC differentiation into neutrophils, eosinophils, lymphocytes and monocytes (research parameters, non-CE/IVD). Microscopic differentiation was performed by counting 200 WBC on cytospin slides. Results: For the 2-part differentiation, Passing Bablok regression and Spearman correlation analysis revealed good agreement with microscopy for percentage of PMN cells ($y = 0.95x + 2.2$, $R = 0.95$) and MN cells ($y = 1.05x - 3.1$, $R = 0.95$). Moreover, the XN 2000 agreed well with microscopic differentiation for % neutrophils ($y = 0.94x + 2.3$, $R = 0.95$) and lymphocytes ($y = 1.02x - 4.0$, $R = 0.94$), but counted less monocytes ($y = 0.71x + 1.1$, $R = 0.92$). Possible explanations for this discrepancy are: 1) inclusion of macrophages in the microscopic monocyte count, while they are excluded by the XN 2000; 2) selective loss of cells during cytocentrifugation; and 3) cell lysis due to delayed sample processing. Eosinophil numbers were too low to perform quantitative method comparison. Conclusions: This study demonstrates that the automated 2-part differential count on Sysmex XN 2000 is highly comparable to manual microscopy. This instrument can serve as a fast and accurate tool to analyze serous fluids and is therefore implemented in our routine laboratory. For extended 4-part WBC differentiation, we found lower monocyte counts on the XN 2000 compared to the manual method. Further research will be needed before clinical implementation of the automated 4-part differential count.


Delphine Labaere\textsuperscript{1}, Nancy Boeckx\textsuperscript{2}, Inge Geerts\textsuperscript{1}, Marc Moens\textsuperscript{1}, Marleen Van den Driessche\textsuperscript{1}

\textsuperscript{1}Imelda Hospital Bonheiden, Belgium, \textsuperscript{2}University Hospitals Leuven, Belgium

Introduction: A fast and accurate differentiation of white blood cells in umbilical cord blood (UCB) has been used as a source of stem cells for cell therapy. In a previous work we have shown that several subsets of CB mononuclear cell (MNCs) were viable up to 96 hours after collection and stored at room temperature. Methods: We analyzed the viability and functionality of these cells before and after cryopreservation. Samples from twenty UCB units were analyzed at 24 and 96 hrs after collection, frozen for 6 months and reevaluated post-thaw. MNCs were analyzed by flow cytometry. Cell viability was examined by 7-AAD. Clonogenic assays were performed pre-freezing and post-thaw. Results: Delay in processing UCBs caused no substantial loss of MNCs (median 7.320x10\textsuperscript{6} x 6.305x10\textsuperscript{6}). Number and viability of CD34\textsuperscript{+} cells, B-cell precursors and mesenchymal stem cells did not decrease significantly. Delay in 96 hours for processing UCB was associated with a relative loss of colony formation (median 12%), but post-thaw, this loss had a median of 49% (samples processed at 24 hours) to 56% (samples processed at 96 hours). Conclusions: A delay of 96 hrs before processing of UCB is possible, without a prohibitive impairment of stem cell loss in number and functionality. Freezing and thawing impairs substantially the stem cell functionality.

Stability for WBC VCSN Parameters

Robert Magari, Fernando Chaves, Aprille Seidel, Liliana Tejidor, Patrick O’Neil

Beckman Coulter Miami, FL, USA

Introduction: The UniCel DxH Series of Coulter Cellular Analysis Systems using VCSN Technology analyzes seven parameter measurements for each leukocyte. Limited information is available on the effect of sample aging on these parameter measurements. Our study investigated the effects of sample aging on the Mean Monocyte Volume (@MN-V-MO), Mean Monocyte Volume Distribution Width (@SD-V-MO), Mean Neutrophil Volume (@MN-V-NE), and Mean Neutrophil Volume Distribution Width (@SD-V-NE) parameters. Methods: The experiment included 21 normal whole blood specimens collected in a salt of EDTA from three sites. Samples were aliquoted and analyzed within 2 hours post collection as time 0. Samples were stored at room temperature and re-analyzed in duplicate at 8, 12, 24, 36 and 48 hours post collection. Responses were modeled as a second degree polynomial function of time while sample-to-sample variability was considered as the random component in the model. Changes for the selected parameter measurements from time zero were calculated for samples at the aforementioned times. Drift was calculated as the difference between the response at time zero and the response from the statistical model at the aforementioned times. Standard error of the drift was based on the standard errors of the polynomial estimates and their covariance. 95% confidence limits of the drift were also calculated. Results: There was no significant change for @MN-V-MO over time. Upward trends were observed for @MN-V-NE, @SD-V-MO and @SD-V-NE. At 12 hours, all parameters excluding @SD-V-NE demonstrated % drift of approximately 10% from time zero based on the upper limit of the 95% confidence range. The results for 8, 12 and 24 hours are included in the table below. Conclusions: The characterization of the stability of the VCSN parameter measurements in aging samples is important for the analytical evaluation of these parameters.

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129 COMPARATIVE STUDY OF BODY FLUID CYTOLOGICAL ANALYSIS BY CONVENTIONAL MICROSCOPY AND USING THE CELLAVISION DM96 FOR MALIGNANCY DETECTION IN THE EMERGENCY LABORATORY

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1Hemotherapy-Hemostasis Department. CDB. Hospital Clinic. Barcelona Spain.

Introduction: We evaluated the Cellavision DM96 (DM96) system for body fluid body fluid image analysis, comparing the results with direct manual microscopy and after additional histochemical and immunocytochemical stains in the Pathology Laboratory. Methods: We analyzed in the Emergency Laboratory a total of 90 body fluids (40 peritoneal, 33 pleural, 15 cerebrospinal and 2 joint) from 90 patients (48 men and 42 women). When the number of cells were >250/μL (or >10/μL for cerebrospinal fluid) we used a cytospin for their cytological evaluation. All the slides were stained with May Grünwald-Giemsa and the percentages of cell subtypes were counted. After the microscopic analysis the slides were loaded into the DM96 obtaining digital images of preclassified cells, which were verified or corrected when necessary (DM96POST). Pathological samples were sent to the Pathology Laboratory in order to confirm the suggested diagnosis based in morphological analysis. Statistical analysis was performed using the correlation (Pearson) test. Results: Correlation coefficients between results obtained from the Cellavision DM96 preclassification and by conventional direct microscopy were good for neutrophils (r: 0.93), eosinophils (r: 0.92) and lymphocytes (r: 0.85), p < 0.0001. For macrophages, the results were not as good (r: 0.681), but correlation was higher when we compared DM96POST values (r: 0.86). Mesothelial cells, as well as atypical cells, were classified only by the cytologist. Evaluation of the body fluid morphology under the microscope reported malignancy in 26/90 cases in which the final diagnosis was malignancy in 19, being the remaining 7 “false positives” (FP). Nevertheless, when we analyze the concordance taking into account the DM96POST we observed malignancy in 15/19 cases. In addition, 3 FP, 2 “false negatives” (FN) and 3 “no valid” (NV) were detected using the DM96, which means that a 21 % of the malignant samples were not detected.

Conclusions: Comparison of morphological classification of body fluid cells by the automated system DM96 showed a good correlation with respect to manual differentials for neutrophils, eosinophils and lymphocytes. With respect to the atypical cell analysis, the images obtained by the system at low magnification were not good enough for their evaluation. When we analyzed them at high magnification one fifth of the pathological samples were not detected. We concluded that the Cellavision DM96 is a good tool for automatic morphological analysis for normal body fluid samples. Nevertheless, we recommend the examination of the body fluids under the microscope to detect malignancy.

131 REFERENCE RANGE STUDY FOR BLOOD CELL POPULATIONS DETECTED WITH CYTODIFF.

Elena V. Naumova, Igor Y. Bugrov, Fedor A. Dukov, Alexander I. Kostin, Margarita E. Pochtar, Svetlana A. Lugovskaya
Russian Medical Academy for Postgraduate Education Moscow, Russia

Introduction: HematoFlow is the new technology for WBC differential utilizing flow cytometric analysis with CytoDiff reagent (5-color/6-marker reagent, Beckman Coulter, Brea, CA, USA). In addition to the five classical populations (Lymphocytes, Monocytes, Neutrophils, Basophils, Eosinophils), this new combination provides the ability to identify and characterize new subsets of white blood cells, hence expanding the first steps in blood analysis. The aim of this study was to obtain the reference ranges for all cellular populations detected with the CytoDiff. Methods: EDTA - anticoagulated venous whole blood was analyzed within 8 hours of collection. 82 blood samples obtained from healthy individuals were stained with the CytoDiff panel (CD36-FITC, (CD2+CD294)-PE, CD19-ECD, CD16-PC5 and CD45-PC7), lysed and 20000 leukocytes were analyzed on a FC500 Flow Cytometer (Beckman Coulter) with CytoDiff CXP software. Reference intervals were calculated using MedCalc software (Ostend, Belgium) with non-parametric percentile method (CLSI C28-A3). Results: Reference intervals for all cell populations detected with CytoDiff are presented in Table 1 for Lymphocyte and Monocyte subpopulations and in Table 2 for Granulocyte subpopulations and for Immature cells (Immature Granulocytes (Immature Gran) and Blasts). The 5th and 95th percentile is shown for each cell type. Only for immature cells both reference ranges – in absolute values and in percentages - have been calculated. For all other populations only reference intervals for absolute count have been analyzed. Table 1
Table 2

<table>
<thead>
<tr>
<th>Population</th>
<th>Low limit</th>
<th>High limit</th>
<th>Population</th>
<th>Low limit</th>
<th>High limit</th>
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<td>Immature Gran, cells/ml</td>
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<td>Eosinophils, cells/ml</td>
<td>42</td>
<td>303</td>
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<tr>
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<td>5388</td>
<td>Basophils, cells/ml</td>
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<td>119</td>
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<td>Neutro Total, cells/ml</td>
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<td>5401</td>
<td>Blasts Total, cells/ml</td>
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<td>32</td>
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<tr>
<td>Immature Gran, %</td>
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<td>0.44</td>
<td>Blasts Total, %</td>
<td>0</td>
<td>0.95</td>
</tr>
</tbody>
</table>

Conclusions: HematoFlow and CytoDiff is new technology for flow WBC differential, allowing to decrease the number of microscopic reviews and to increase the objectivity and reliability of the results. New cellular populations detected with CytoDiff – lymphocyte and monocyte subpopulations and immature cells – can provide additional important information about the patient. Determination of reference ranges on the group of healthy individuals is the first step necessary for the introduction of new technology in routine hematological laboratory.

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MARROW FLUID TESTING ON MINDRAY BC-6800 HEMATOLOGY ANALYZER: FORMULATING A SCORE SYSTEM BASED ON BASIC HEMATOLOGY PARAMETERS, TO DISCRIMINATE MYELODYSPLASTIC SYNDROMES (MDS) FROM REACTIVE MARROW
Georgios Paterakis1, Georgios Oudatzis2, Maria Simiri3, Sara Tryfonidou4, Paraskevi Vassileiou5

Introduction: INTRODUCTION: Diagnosis of myelodysplastic syndromes (MDS), based on subjective morphology assessment of bone marrow smears, has been supported by multiparameter bone marrow flow cytometry by implementing scores on several cell subpopulation phenotypic aberrations. The contemporary Mindray automated hematology analyzer BC-6800, due to certain inherent features like freedom from lipid interference, availability of very high linearity of counts in increased white cell–lymphocyte and monocyte subpopulations and immature cells – can provide additional important information about the patient. Determination of reference ranges on the group of healthy individuals is the first step necessary for the introduction of new technology in routine hematological laboratory.

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EVALUATION OF THE CAPABILITY OF BODY FLUID CELL COUNTING AND LEUKOCYTE DIFFERENTIAL OF THE UNICEL DXH 800 ANALYZER
Ines Rodriguez-Hernández1, Marina Carbonell1, Alba Leis2, Amparo Galan2, Maria-Cruz Pastor2, Evarist Felii3, Fuensanta Millà4

Introduction: Cell differentiation on body fluids constitutes a valuable tool, as it permits to characterize the liquid and, therefore, aid to diagnosis. Manual methods are labor-intensive and imprecise, so the capabilities of several automated hematology analyzers have been extended to include body fluid cell counting. The aim of this study was to compare manual hemocytometer with new Unicel DXH 800 (DXH) in cell counting of several of body fluids. Methods: During a 3 week period, we consecutively processed all body fluids sent to the laboratory to cell count by the DXH 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. In order to achieve a valuable comparison of leukocyte differentiation, we performed a cyto centrifugation and May Grünwald-Giemsa staining to be counted by 2 different expert cytologists, and if it is available, the rest of sample was assessed for cell identification by flow cytometry, which included antibodies against CD15/CD14/CD3/CD19/CD45. Passing and Bablock regression and Spearman correlation were used for method comparison. Results: Forty-six consecutive liquids sent to the laboratory to cell count were included, 7 out of which were pleural, 20 peritoneal, 13 cerebrospinal (CSF) and 6 synovial fluids. Fifteen out of them (7 pleural, 5 peritoneal, 4 CSF, 1 synovial) could not be compared because of too little amount or bad conditions of the sample. A variation coefficient between observers with Neubauer camera was between 0 and 34 in RBC counts, and 0 to 60 in leukocyte counts. For erythrocyte counting, systematic differences were observed when comparing manual and DXH methods, but there were no proportional differences, with a positive test of linearity with Passing and Bablok method [Y = 278.8 + 1.25X]. The Spearman correlation was r = 1.0. For leukocyte counting, neither systematic differences nor proportional ones were observed with a positive test of linearity with Passing and Bablok method [Y = 13.4 + 1.06X], r = 0.90. Differential leukocyte count could only be assessed in 2 samples, as we initially performed the CBC mode in DXH, which is not optimal. We are currently analyzing it properly. Conclusions: Manual and automated DXH cell methods for counting pleural, peritoneal, CSF and synovial fluids were interchangeable.
DXH body fluid cell count constitutes a valuable, quick tool for laboratories.

**137 NEUT X AND NEUT Y: ROLE IN MULTIPARAMETRIC APPROACH TO MDS DIAGNOSIS.**

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**Introduction:** Neut X and Neut Y are newer parameters in XE series of Sysmex analyzers. There are seen to be significantly lower in myelodysplastic syndromes. Neut X is an indicator of the side scatter of neutrophils, while Neut Y is an indicator of the side scatter of lymphocytes. The peripheral blood was run in the bone marrow examination, immunohistochemistry, cytogenetics laboratories. Many patients with MDS have macrocytic indices and can be confused with megaloblastic anemias. Many patients with MDS have macrocytic indices and can be confused with megaloblastic anemias.

**Methods:** Four groups were studied. These included 66 controls came for regular health check ups, 30 patients with proven MDS (Myelodysplastic syndrome), 56 patients with MDS, MPN and megaloblastic anemias and to determine biochemical parameters. The peripheral blood was run in the retic mode of Sysmex XE 2100 and the values of Neut X and Y calculated.

**Results:** Neut X showed a significant decrease in MDS, and thus can serve as an important screening tool in evaluation of MDS patients. This can guide smear review rates to pick up MDS cases on screening. Neut Y in our study was not statistically significant. MPN's showed no difference from control group. However, this could be brought to use in cases where there is morphologic overlap between MDS and MPN groups. Interestingly megaloblastic anemias showed significant increase in both Neut X and Y in our study. This too can help since many patients with MDS have macrocytic indices and can be confused with megaloblastic anemias.

**Conclusion:** Neut X showed a significant decrease in MDS, and thus can serve as an important screening tool in evaluation of MDS patients. This can guide smear review rates to pick up MDS cases on screening. Neut Y in our study was not statistically significant. MPN's showed no difference from control group. However, this could be brought to use in cases where there is morphologic overlap between MDS and MPN groups. Interestingly megaloblastic anemias showed significant increase in both Neut X and Y in our study. This too can help since many patients with MDS have macrocytic indices and can be confused with megaloblastic anemias.

**139 THE EFFECT OF DABIGATRAN ON LABORATORY TESTING: RESULTS FROM THE RCPAQAP HAEMATOLOGY TESTING:**

John Sioufi1, Roslyn Bonar1, Emmanuel Favaloro2, Katherine Marsden1

1RCPAQAP Pty Limited Sydney Australia, 2Westmead Hospital, NSW, Australia

**Introduction:** The new direct oral anticoagulants (DOACs) are direct inhibitors of either thrombin (e.g. dabigatran) or activated factor X (FXa) (e.g. rivaroxaban, apixaban). These anticoagulants have recently become available for the prevention and treatment of thrombosis. Although these agents have been developed without the need for laboratory monitoring, there may be situations in which assessment by laboratory testing is useful. The aim of this exercise was to collect and assess recent information from laboratories performing dabigatran testing and to survey current test practices.

**Methods:** 72 laboratories were sent between 1-3 sets of 7 samples spiked with Dabigatran. The level of Dabigatran ranged from 0-800ng/mL. Specifically, one set of 7 samples was issued for each testing batch scenario according to testing of (A) Routine Coagulation tests (e.g., PT, APTT, TT); (B) Specific tests (e.g., for dabigatran: Hemoclot, dTT, ECT, dRVVT, PICT, anti-IIa); (C) Drug Interferences (e.g., LA tests, Protein C, S or Antithrombin deficiencies, anti-Xa, etc); (D) Other tests (e.g., thrombin generation, thromboelastography) with a maximum of 3 sets per participant. **Results:** 70 (97%) laboratories returned completed results. There was excellent correlation between the target level, the Hemoclot and in house Dilute Thrombin Time results. Many of the routine assay such as the PT, APTT, thrombin time and factor assays, for example, were adversely affected by increasing levels of dabigatran in the samples provided. Selected results for the PT, APTT, TT and FVIII assays are presented in Table 1. Table 1. **Snapshot of results from selected assays and Dabigatran concentrations**

<table>
<thead>
<tr>
<th>Dabigatran conc</th>
<th>0</th>
<th>10 ng/mL</th>
<th>36 ng/mL</th>
<th>86 ng/mL</th>
<th>190 ng/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>PT (secs)</td>
<td>15</td>
<td>16.3</td>
<td>16.6</td>
<td>17.9</td>
<td>19.9</td>
</tr>
<tr>
<td>APTT (secs)</td>
<td>38.5</td>
<td>47.7</td>
<td>54.5</td>
<td>64.0</td>
<td>77.8</td>
</tr>
<tr>
<td>TT (secs)</td>
<td>18.4</td>
<td>73.25</td>
<td>118.8</td>
<td>150.1</td>
<td>135.1</td>
</tr>
<tr>
<td>FVIII (%)</td>
<td>48.5</td>
<td>41.85</td>
<td>36.95</td>
<td>26.5</td>
<td>15.0</td>
</tr>
</tbody>
</table>

C 2014 The Authors
FIX (%)  75.0  63.0  54.0  39.0  22.5

Conclusions: This exercise has provided valuable data to show the sensitivity of the routine tests for measuring the presence of dabigatran and which test would be most useful in providing this information.

141 AUTOMATED CEREBROSPINAL FLUID CELL COUNTS IN THE SYSMEX XN-SERIES

Patricia Tejerina, Maite Serrando, Jose Manuel Ramirez
Clinical Laboratory, Hospital Josep Trueta Girona, Spain

Introduction: The analysis of cerebrospinal fluid (CSF) samples is an essential tool in clinical practice. The reference method used for cell counting is the manual cytometric chamber. The development of automated systems for the analysis of the CSF decreases interoperator variability and this process is standardized, however it’s important that the analyser is able to perform in samples with low cell count. The objective of our study was to compare manual with automated white blood cell count on CSF.

Methods: 122 samples from adults (101) and children (21) were examined. The samples were obtained from puncture lumbar and ventriculars (shunt)(20). The cellular count was processed with a Sysmex XN-class (XN-10) haematology analyzer and the Neuvaber chamber as a reference method. The detection limit was 0 cells /µl from XN and 5 cells / µl by manual method. The lineal correlation, the passing Bablock and descriptive distributions of values were used for the statistical analysis.

Results: Manual WBC ranges and corresponding Sysmex 95Th percentil ranges.

<table>
<thead>
<tr>
<th>n cases</th>
<th>Manual WBC range</th>
<th>Sysmex 0-95 percentile range</th>
<th>Manual median</th>
<th>Sysmex median</th>
</tr>
</thead>
<tbody>
<tr>
<td>82</td>
<td>0-5</td>
<td>0-21</td>
<td>1.8</td>
<td>3.3</td>
</tr>
<tr>
<td>93</td>
<td>0-10</td>
<td>0-28</td>
<td>3.0</td>
<td>4.25</td>
</tr>
<tr>
<td>22</td>
<td>6-20</td>
<td>1-30</td>
<td>13.2</td>
<td>12.7</td>
</tr>
<tr>
<td>38</td>
<td>6-400</td>
<td>1-462</td>
<td>54.7</td>
<td>61.7</td>
</tr>
</tbody>
</table>

The specify XN10 was 88%, 13 samples (9 pediatric samples i/o 7 shunts samples) were classified as pathological by the automated method with a manual cell count lower than the reference range value. The sensibility was 92%, all the wrong classified samples shown manual cell counts between 10-20 cell / µl whereas the sensibility was 100% for cell counting >20 cell / µl. The Passing Bablok results for the range counting 6-400 were: correlation coefficient, r=0.94; slope 1, 34 ( [.IC] 1.2.1.650); intercept -5.4 (-13.8 to -2.1). The distribution of the data suggests that the relationship between the 2 methods might not be linear in the low and grey range. Conclusions: Although most patients whit low and high cell counts are correctly classified, the automated counting is higher than the chamber method. This fact is important in the cells count near the non pathologic reference values so many authors suggest that a review of the reference values would be useful. Our preliminary data suggested that additional studies would be necessary in pediatric and ventriculars samples.

143 RETICULOCYTE HB EQUIVALENT AND HYPOCHROMIC RED CELLS IN THE STUDY OF ERYTHROPOIESIS IN PREGNANCY

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1HOSPITAL GALDAKAO USANSOLO GALDAKAO VIZCAYA, Spain, 2Department of Pharmacology and Physiology Faculty of Medicine. University of Zaragoza. Zaragoza, Spain

Introduction: Anemia is a common problem during pregnancy, iron deficiency anemia is the predominant cause in those subjects. Due to physiological alteration of plasma volume and red cell mass in pregnancy a slightly reduced Hemoglobin values can be observed, and remains a challenge to decide between physiological hemodilution and pathological status due to iron restricted erythropoiesis. Erythrocite and reticulocyte extended parameters by the Sysmex XE 5000 (Sysmex Corporation, Kobe, Japan) reflect the bone marrow activity and the erythropoiesis status. The aim was to assess the reliability of reticulocyte Hb equivalent (RetHe) and percentage of hypochromic erythrocytes (%HypoHe), in the detection of iron deficient erythropoiesis in women in the second trimester of pregnancy. Methods: Seventy women (23-40 years, mean 33 y) were included in the study; the analyses had been requested by general practitioners, during the routine control in the 20th week of pregnancy. Samples were analyzed for full blood count and reticulocytes within 6 hours of collection; serum ferritin, serum iron and transferrin saturation (Tf Sat ) were measured. Independent samples t test was performed in order to detect statistical deviations between the groups of patients; p values less than 0.05 were considered to be statistically significant. The diagnostic performance of the standard and extended parameters was evaluated with Receiver operating characteristic (ROC) curves; Transferrin saturation <15% was the gold standard for iron restricted erythropoiesis detection. Results: Based on Tf Sat values 20 the pregnant (28.6 %) suffered iron deficiency for erythropoiesis; ferritin, MCH, MCV, RetHe and %Hypo values in both groups were statistically different, P<0.001; ROC analysis is summarized in the table. The definition of anaemia during pregnancy was based on the recommendation of the World Health Organization (Hb < 110 g / L); the cut-off points for iron status measurements were selected on the basis of the reference intervals of the American College of Obstetricians, published on www. update.com. The cut offs for RetHe and %HypoHe were those which provide the best diagnostic performances.

<table>
<thead>
<tr>
<th></th>
<th>AUC</th>
<th>95% CI</th>
<th>CutOff</th>
<th>Sensitivity %</th>
<th>Specificity %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb</td>
<td>0.69</td>
<td>0.59-0.79</td>
<td>110 g/L</td>
<td>52.6</td>
<td>80.9</td>
</tr>
<tr>
<td>MCH</td>
<td>0.71</td>
<td>0.61-0.81</td>
<td>30 pg</td>
<td>65.5</td>
<td>81.1</td>
</tr>
<tr>
<td>MCV</td>
<td>0.69</td>
<td>0.59-0.79</td>
<td>85 fL</td>
<td>42.5</td>
<td>80.2</td>
</tr>
<tr>
<td>RetHe</td>
<td>0.79</td>
<td>0.70-0.88</td>
<td>29.9 pg</td>
<td>80.7</td>
<td>71.3</td>
</tr>
<tr>
<td>%Hypo</td>
<td>0.75</td>
<td>0.65-0.83</td>
<td>1.5 %</td>
<td>82.3</td>
<td>68.3</td>
</tr>
</tbody>
</table>

Conclusions: RetHe and %HypoHe reflect iron deficient erythropoiesis, could be reliable parameters for detecting iron deficiency during pregnancy and useful indicators to identify patients who will benefit from therapy.
DYSPLASTIC BONE MARROW FEATURES CAN BE PREDICTED USING THE ERYTHROGRAM PROVIDED BY ADVIA 2120I ANALYZER

Diego Velasco-Rodríguez, Juan Manuel Alonso-Domínguez, Carolina Muñoz-Novas, Pilar Massó, Marta Jiménez-Rolando, Virginia Quirós, Natalia Acedo, Cristina Seri, Raquel Guillén, Beatriz Alvarez, Fernando Atalúño González-Fernández, Fernando Cava, Jesús Villarrubia
1Laboratorio Central de la Comunidad de Madrid (Unilabs, BR Salud) Madrid, Spain, 2Hospital Infanta Leonor Madrid, Spain, 3Hospital Infanta Sofia San Sebastián de los Reyes (Madrid), Spain, 4Hospital del Henares Coslada (Madrid), Spain, 5Hospital Infanta Cristina Parla (Madrid), Spain

Introduction: Although haematological analyzers provide crucial information for the diagnosis of several types of anemia (thalassaemia, iron deficiency anemia, hereditary spherocytosis), their utility in identifying myelodysplastic syndromes (MDS) is not well defined. Rovó et al demonstrated that sideroblastic changes in bone marrow can be predicted with a characteristic pattern of the erythrogram provided by Advia 2120i analyzer (Siemens Diagnostics®). The mentioned pattern shows unusual broad scatter distribution of the red cells in respect of their volume and hemoglobin concentration, occupying 8 of the 9 areas of the erythrogram (Figure 1).

Methods: Prospective and descriptive study of 16 cases of macrocytic anemia with anisocytosis with erythrogram suggestive of sideroblastic changes in the bone marrow (Rovó et al). Haematological (classical parameters) and biochemical (ferritin, LDH) data were collected. The following variables from the bone marrow aspirate were evaluated: presence of dyserythropoiesis, presence of >15% ring sideroblasts, dysgranulopoiesis, dysthrombopoiesis, excess of blasts and cytogenetic alterations. Final diagnosis according to WHO criteria was made.

Results: Age: 77.06 years (64-87). Gender (M/F): 9/7. Haematological parameters: Hemoglobin 10.88 g/dL (9.12-6.6), MCV 103.49 fl (88.1-119.4), MCHC 31.17 g/dL (29-33.2), RDW 22.83 (20.50-26.30), leukocytes 8.81 x1000/μL (1.41-46.83), platelets 359.6 x1000/μL (71-703). Biochemical parameters: LDH 255.2 (134-552), Ferritin 522.35 ng/mL (58-2048). Final WHO diagnosis: 3 Simple Refractory Anemia (SRA), 3 Refractory Anemia with Ring Sideroblasts (RARS), 4 RARS with thrombocytosis, 3 Refractory Cytopenia with Multilineage Dysplasia (RCMD), 1 Refractory Anemia with Excess of Blasts (RAEB) type 1, 1 unclassifiable MDS and 1 Myelofibrosis. Ten subjects (62.5%) presented with >15% ring sideroblasts in the bone marrow. Cytogenetic alterations: 12 subjects had normal karyotype, 1 presented with -Y, 1 with inv(7), 1 with del(20q) and del(3q), and dry tap was found in 1 patient (myelofibrosis).

Conclusions: Although not all MDS show a typical pattern, there is actually a characteristic pattern in the erythrogram provided by Advia 2120i analyzer that predicts dysplastic bone marrow features. This can be really helpful in daily practice for patients with macrocytic anemia and anisocytosis, since dysplastic changes can be assessed in the analysis of the blood smear and a bone marrow aspirate can be recommended in these patients. The proportion of patients with >15% ring sideroblasts in the bone marrow was lower than the proportion reported by Rovó et al. Clinical applicability of these results is still to be determined, and they need to be validated in studies with larger number of people.

EVALUATION OF THE UTILITY OF THROMBOELASTOGRAPHY (TEG) IN A TERTIARY TRAUMA CARE CENTRE

Venecia Albert, Arulselvi Subramanian, Deepak Agrawal, Renu Saxena, Ravindra Mohan Pandey
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Introduction: Coagulopathy following trauma is associated with increased morbidity and mortality in trauma patients. Thromboelastography (TEG) unlike conventional coagulation assays evaluates the dynamic interaction of clotting factors and platelets indicating an overall clot quality. Literature assessing the efficacy of TEG in identifying the etiology of trauma associated bleeding is lacking. We aimed to compare TEG with conventional plasma based routine coagulation tests and assess whether TEG can serve as a screening test or replace the conventional routine test for the detection of trauma coagulopathy.

Methods: Retrospective data was collected for 150 severe trauma patients. Patients with known evidence of severe comorbidities, which may influence the outcome were excluded. Detailed evaluation of the patient’s clinical and laboratory records was conducted. Diagnostic characteristics such as sensitivity, specificity, and accuracy were described in terms of true positive (TP), true negative (TN), false negative (FN), and false positive (FP).

Results: Fifty-one patients were defined as coagulopathic by the conventional coagulation test, 30 by the laboratory established range for TEG were negative (TN), false negative (FN), and false positive (FP).

Conclusions: Although not all MDS show a typical pattern, there is actually a characteristic pattern in the erythrogram provided by Advia 2120i analyzer that predicts dysplastic bone marrow features. This can be really helpful in daily practice for patients with macrocytic anemia and anisocytosis, since dysplastic changes can be assessed in the analysis of the blood smear and a bone marrow aspirate can be recommended in these patients. The proportion of patients with >15% ring sideroblasts in the bone marrow was lower than the proportion reported by Rovó et al. Clinical applicability of these results is still to be determined, and they need to be validated in studies with larger number of people.
Introduction: Thrombelastometry offers a true vision of the Hemostatic status situation and allows to differentiate the causes of abnormalities related to coagulopathy, specially bleeding disorders and the use of anticoagulants. Its applicability is the monitoring of critical patients during surgical situations of liver and cardiac transplantation and in subsequent monitoring. Coagulation factors deficiencies can be discriminated from the effects of anticoagulants when used specific reagents, as well as, the conditions of hyperfibrinolysis and platelet dysfunction. The reagents are INTEM® that evaluates intrinsic pathway; EXTEM®, the extrinsic pathway and FIBTEM® the polymerization of fibrin problems enabling the differentiation with the blood platelet disorders. Our objective was to correlate the kinetic and structural layouts parameters of thrombelastometry with the results of coagulation. Methods: Was evaluated 68 thrombelastometry profiles from critical patients processed on equipment ROTEM®-DELTA. The kinetic and structural related respectively to EXTEM, INT EM® and FIBTEM® reagents and were compared with PT, APTT, TT and fibrinogen obtained by coagulometric methodology in the equipment STA-R Evolution-STAGO® and platelet count impedance by equipment Sysmex® XE2100. Results: INTEM® was concordant in 92.3% between hypokinetic curve and APTT results, and 32.7% with between the normal results. EXTEM® was concordant in 88.9% between hypokinetic curve and TP results, and 53.8% between the normal results. With the FIBTEM® there were 89.5% of correlation between structure and hypofibrinogenemia, 58.3% between the normal results and 90.0% presented hyperstructure and hyperfibrinogenemia. Conclusions: We could conclude that there is wide concordance among evaluated methodologies with the advantage of thrombelastometry to have short time of release and allow immediate therapeutic action.

207 QUANTIFICATION OF RIVAROXABAN: EVALUATION OF 2 CHROMOGENIC ASSAYS COMPARED TO ULTRA-PERFORMANCE LIQUID CHROMATOGRAPHY TANDEM-MASS SPECTROMETRY IN RIVAROXABAN TREATED PATIENTS

Anthony FOURIER, Lucia RUGERI, Marie Claude GAGNIEU, Micheline BERRUYER, Claude NEGRIER Hospices Civils de Lyon Lyon, France

Introduction: Rivaroxaban is a novel oral direct factor Xa inhibitor licensed for prophylaxis and curative treatment of venous thromboembolism and for prophylaxis of ischemic stroke in patients with atrial fibrillation. The aim of the study was to assess 2 chromogenic assays for the determination of rivaroxaban concentration in treated patients in comparison to validated ultra-performance liquid chromatography (UPLC) tandem-mass spectrometry method. Methods: Patients admitted in orthopedic or in neurologic units and receiving a prophylaxis treatment with rivaroxaban (10 or 20 mg once daily) were included into a non-interventional trial. Samples of 21 patients were collected in serum tubes (chromatographic assay) and in citrate tubes (chromogenic
assays) 2 hours after the uptake of rivaroxaban (peak concentration) and/or at trough concentration time. Plasma/sera were centrifuged and frozen at -20°C until analysis. The chromatographic assay was performed on a Nexis UPLC-TQ MS (Waters, France), and validated according to ISO 15189 and EMEA recommendations. Standard curves were constructed using 7 levels of calibration between 0 and 600 ng/mL. Both anti-Xa chromogenic assays, Biophen Heparin LRT and BiophenDiXaI (HyphenBioMed, France) were performed on a hemostasis analyzer ACL-TOP (Instrumentation Laboratory, USA), with specific rivaroxaban standard calibrators and controls (Hyphen BioMed). Heparin LRT assay is suitable for direct and indirect factor Xa inhibitors including heparins, whereas DiXaI assay is specific to direct factor Xa inhibitors. Results: The measurement of rivaroxaban showed a large range of concentrations, from 2 to 417 ng/mL, reflecting the variety of doses and time measurements (peak and trough). Table 1 describes results obtained with the 3 different assays. Using a Bland-Altman representation, a good agreement between both chromogenic assays and UPLC was observed.

Using Pearson test, Heparin LRT and DiXaI assays showed a good correlation with the UPLC quantification in treated patients (Figure 1). An underestimation of rivaroxaban concentrations was observed with chromogenic assays, probably explained by the dilution of plasma collected in citrate tubes.

Conclusions: Despite the highest biases observed with low concentrations < 30 ng/mL, especially for DiXaI assay, both chromogenic assays provide reliable methods for the measurement of therapeutic concentrations of rivaroxaban.

<table>
<thead>
<tr>
<th>n = 21</th>
<th>Heparin LRT</th>
<th>DiXaI</th>
<th>UPLC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median (ng/mL)</td>
<td>45</td>
<td>56</td>
<td>60</td>
</tr>
<tr>
<td>Minimum (ng/mL)</td>
<td>5</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Maximum (ng/mL)</td>
<td>311</td>
<td>353</td>
<td>417</td>
</tr>
<tr>
<td>Median bias vs UPLC (%)</td>
<td>-17</td>
<td>-5</td>
<td>NA</td>
</tr>
</tbody>
</table>

1) Prolonged PiCT® M4: presence of DOAC. 2) Normal PiCT® M1 and prolonged PiCT® M4: presence of direct Xa-inhibitor.
3) Prolonged PiCT® M1 and M4: presence of a FIIa-inhibitor. 4) The second operator correctly determined the nature of DOAC contained in the 10 blinded samples. Conclusions: Pefakit® PiCT® demonstrates potential as tool in detection of and discrimination between DOACs. Combination of PiCT®, PT and aPTT could further discriminate other ACs (data not shown). Its applicability in the clinical environment will be challenged with pathological samples from patients undergoing DOAC therapy. References: 1: Calatrazis A. et al. Am. J. Clin. Pathol 2008;130:446-454. 2: Korte W. et al. Haemostaseologie 2010;4:212-216.

209 PEFAKIT® PiCT® AS DOAC IDENTIFICATION TOOL.
Dominique Haldemann¹, Erik-Jan van den Doo², Abel Ferrándiz³, An Stroobants², Anne Brisset¹
¹DSM Branch Pentapharm Aesch, Switzerland, ²Laboratory for General Clinical Chemistry, Academic Medical Centre, University of Amsterdam Amsterdam, Netherlands

Introduction: Direct oral anticoagulants (DOACs) have been proved effective and safe without a need for dose adjustment or recurrent monitoring. However, physicians seek methods confirming the presence and nature of DOACs in plasma samples to deal with emergency situations. Pefakit® PiCT® is a functional assay which has been demonstrated responsive to all direct or indirect anti-Xa or anti-thrombin anticoagulants in the market 1-2. different methodologies offer a different level of responsiveness to different anti coagulants (ACs). The present study aims to analyze the power of Pefakit® PiCT® as universal tool to determine presence and discriminate the nature of DOACs in a plasma sample taking advantage of different responsiveness to different methods. Methods: Pefakit® PiCT® ranges were established in plasma samples from healthy individuals using 2 methodologies: M1 and M4. Plasma samples with and without spiked DOACs were evaluated with Pefakit® PiCT® M1 and M4 methods on an ACL TOP® 500 to establish clotting time patterns. Clotting times superior to the upper limit of reference range were considered prolonged. Ten tubes filled with blank plasma or plasma containing one particular DOAC were prepared by an operator and subjected to the 2 aforementioned tests by a second operator blinded to their contents. The second operator was asked to determine the content of the blinded tubes. Results:

<table>
<thead>
<tr>
<th>PiCT®M1 (s)</th>
<th>PiCT®M4 (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference ranges</td>
<td>24.4-30.7</td>
</tr>
<tr>
<td>Blank plasma</td>
<td>25</td>
</tr>
<tr>
<td>Dabigatran (20 and 300 ng/mL)</td>
<td>37 and 117</td>
</tr>
<tr>
<td>Rivaroxaban (20 and 300 ng/mL)</td>
<td>17 and 29</td>
</tr>
<tr>
<td>Apixaban (20 and 300 ng/mL)</td>
<td>18 and 25</td>
</tr>
</tbody>
</table>

211 PREDICTION OF SURVIVAL IN PATIENTS WITH SUSPECTED DISSEMINATED INTRAVASCULAR COAGULATION USING ANTITHROMBIN, PROTEIN C, ANTIPLASMIN AND ISTH SCORE.
Einar Hjorleifsson¹, Martin Sigurdsson¹, Brynja Gudmundsdottir¹, Gisli Sigurdsson¹², Pall Onundarson¹²
¹University of Iceland School of Health Sciences, Faculty of Medicine Reykjavik, Iceland, ²Landsstílaháskóli National University Hospital Reykjavik, Iceland, ³Brigham and Womens Hospital Boston, MA, USA

Introduction: Although antithrombin, protein C and antiplasmin are consumed during overt disseminated intravascular coagulation (DIC), their association with morbidity and mortality in critically ill patients not fulfilling criteria for DIC is not as well defined. Methods: All patients suspected of DIC during a 5 year period at a single tertiary care institution were identified and assessed retrospectively for mortality in relation to their antithrombin,
protein C and antiplasmin activity that was measured as a part of the DIC screening panel and their ISTH score. Those scoring ≥5 (DIC group) and a matched non-DIC subgroup scoring < 5 were assessed for co-morbidity. Results: There were 1826 patients included, 92 had an ISTH score >4 fulfilling ISTH criteria for overt DIC. The one year mortality was 4, 18, 24, 35, 54 and 64% with an ISTH score of 0, 1, 2, 3, 4 and ≥5, respectively. Antithrombin and protein C correlated inversely with the ISTH score (p<0.001) and APACHEII score (p<0.001). Antiplasmin did not correlate with the APACHEII score but decreased when an ISTH score of >3 was reached. Both 28 day and one-year mortality increased progressively as antithrombin and protein C decreased. Conversely, one-year mortality was lower in those with lowered antiplasmin.

Conclusions: Morbidity and mortality increases progressively across the spectrum of severity of the ISTH score in unslected patients suspected of DIC. Decreased antithrombin and protein C also predict worse outcome. Conversely, lowered antiplasmin is associated with improved one-year survival possibly indicating a favorable role of hyperfibrinolysis in these patients. The ISTH criteria for overt DIC may be too strict to identify many patients with substantial mortality.

213 CLOPIDOGREL RESPONSIVENESS AND PLATELET ACTIVATION MARKERS (BETA-THROMBOGLOBULIN AND PLATELET FACTOR 4) IN MYOCARDIAL INFARCTION PATIENTS RECEIVING PERCUTANEOUS CORONARY INTERVENTION
Jimyung Kim, Jinok Jung, Kyechul Kwon, Sunhoo Koo
Chungnam National Hospital Daejeon, South Korea

Introduction: Dual antiplatelet therapy (aspirin and clopidogrel) has been used for the prevention of adverse cardiac events in patients with myocardial infarction (MI) and following percutaneous coronary intervention (PCI). Clopidogrel inhibits platelet activation blocking the P2Y12 ADP receptor and some patients do not have an adequate response. Beta-thromboglobulin (beta-TG) and platelet factor 4 (PF-4) are markers of platelet activation and many studies have reported that platelet activity is increased in MI. We assessed that clopidogrel response is related to dynamics of beta-TG and PF4 concentrations. Methods: Thirty-six patient underwent PCI due to MI and indicated for dual antiplatelet therapy were included in the study. Platelet reactivity using the VerifyNow assay was measured on the 3rd day of PCI and a clopidogrel loading dose. The plasma beta-TG and PF4 concentrations were quantified using the immunoenzymatic method in patients with myocardial infarction (MI) and following percutaneous coronary intervention (PCI). Clopidogrel inhibits platelet activation blocking the P2Y12 ADP receptor and some patients do not have an adequate response. Beta-thromboglobulin (beta-TG) and platelet factor 4 (PF-4) are markers of platelet activation and many studies have reported that platelet activity is increased in MI. We assessed that clopidogrel response is related to dynamics of beta-TG and PF4 concentrations.

Results: The median of P2Y12 reactivity unit (PRU) and percentage inhibition (PI) was 120 (5-379) and 61% (2-98%). Among 36 patients, 15 (41.7%) had less than 50% of PI and 12 (33.3%) more than 200 PRU. On admission, levels of beta-TG, as compared to those in healthy subjects (22.1±7.7 ng/mL), were significantly elevated in patients (45.8±12.2 ng/mL, P<0.0001); 3rd and 10th day of PCI, values were also higher (50.8±18.6 mg/mL, P<0.0001; 30.2±10.8 mg/mL, P=0.006). However, the levels of PF4 showed no significant difference compared to values in healthy controls. The plasma beta-TG levels were decreased significantly on 10th day, but PF4 levels did not show any significant changes during the follow up. Patients with 50% PI or more had lower beta-TG on 10th day compared to those with less than 50% of PI (27.8±8.5 vs 33.6±13.2 ng/mL, P=0.06). Conclusions: We concluded that a change of beta-TG reduction may be related to clopidogrel responsiveness.

215 WARFARIN DOSAGE RESPONSE RELATED PHARMACOGENETICS IN CHINESE POPULATION
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Introduction: As the most frequently prescribed anticoagulant, warfarin has large interindividual variability in dosage. Genetic polymorphisms could largely explain the differences in dosage requirement. rs9923231 (VKORC1), rs7294 (VKORC1), rs1057910 (CYP2C9), rs2108622 (CYP4F2), and rs699664 (GGCX) involved in the warfarin action mechanism and the circulatory vitamin K were selected to investigate their polymorphism characteristics and their effects on the pharmacodynamics and pharmacokinetics of warfarin in Chinese population. Methods: 220 patients with cardiac valve replacement were recruited. International normalized ratio and plasma warfarin concentrations were determined. 5 genetic polymorphisms were genotyping by pyrosequencing. The maintenance dose and plasma concentration differences between the groups categorized by genotypes as well as the relationships of maintenance dose and plasma warfarin concentration, plasma concentration and INR were assessed. Results: The genotype distributions of the tested SNPs obtained in 220 subjects were all in Hardy-Weinberg equilibrium. rs9923231 and rs7294 in VKORC1 had the analogous genotype frequencies (D’: 0.969). 158 of 220 recruited individuals had the target INR (1.5-2.5). Patients with AA of rs9923231 and CC of rs7294 required a significantly lower maintenance dose and plasma concentration than those with AG and TC, respectively (maintenance dose: rs9923231: 19.21±5.66 mg/w vs 28.6±8.02 mg/w, P=0.00; rs7294: 19.40±7.5 7 mg/w vs 27.87±8.80 mg/w, P=0.00; plasma concentration: rs9923231: 117.29±32.23 mg/w vs 167.73±43.10 mg/w, P=0.00; rs7294: 117.29±32.23 mg/w vs 167.73±43.10 mg/w, P=0.00 ). The mean weekly maintenance dose was also significantly lower in CYP2C9 rs1057910 mutated heterozygote than in patients with the wild homozygote (15.31±5.26 mg/w vs 21.21±6.98 mg/w, P=0.00). For patients with allele G of rs9923231 and allele T of rs7294, higher plasma concentration was needed to achieve the similar goal INR. Conclusions: By exploring the relevant genetic variants, this study contributes to a broader and richer insight into the pharmacodynamics and pharmacokinetics of warfarin in Chinese population. A better understanding of the genetic variants in individuals can be the foundation of warfarin dosing algorithm and facilitate the reasonable and effective use of warfarin.

217 ANALYTICAL PERFORMANCE OF HEMOSIL D-DIMER HS 500 TEST AND ITS COMPARISON WITH VIDAS DD EXCLUSION
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Grupo Bioquímico. Laboratorio Central. Hospital Italiano Buenos Aires, Argentina

Introduction: D-dimer testing is widely used in conjunction with clinical pretest probability for venous thromboembolism (VTE) exclusion, as well as recurrence predictor after anticoagulation discontinuation and marker of fibrin formation in DIC score. The aim of the study was to evaluate the analytical performance of an immunonephelometric assay [HemosIL D-Dimer HS 500 (DD...
Poster Presentation Abstracts

219 EVALUATION OF AN AUTOMATED LATEX IMMUNOASSAY FOR VON WILLEBRAND FACTOR ANTIGEN ADAPTED FOR THE STA-R EVOLUTION
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2Department of Medicine, McMaster University Hamilton, ON, Canada.
3Department of Pathology and Molecular Medicine, McMaster University Hamilton, ON, Canada.

Introduction: The von Willebrand factor antigen (VWF:Ag) assay is used for the quantification of von Willebrand factor and is one of a panel of laboratory screening assays used for the investigation of von Willebrand disease. A commonly performed assay for VWF:Ag is the latex immunoassay (LIA) methodology. The LIA method involves the use of latex particles coated with an antibody to VWF:Ag. Plasma added to the beads causes agglutination which is proportional to the VWF:Ag. Most clinical laboratories perform assays using kits and instruments from the same manufacturer. Our laboratory evaluated an adapted latex immunoassay for VWF:Ag, HemosIL von Willebrand Factor Antigen (IL VWF:Ag), previously only validated for IL coagulation systems, run on a Diagnostica Stago STA-R Evolution (STA-R) analyzer. For the evaluation, data were compared for the IL VWF:Ag and the Stago VWF:Ag assay (Stago VWF:Ag), both run on the STA-R. Methods: The IL VWF:Ag assay protocol for the STA-R was developed and validated. Standard curves for both assays were prepared using Diagnostica Stago’s VWF Calibrator which is referenced against WHO VWF:Ag Standard. Samples tested included: 100 healthy volunteer plasmas (blood group not known), 71 patient samples and lyophilized normal and abnormal quality control (QC) samples (Siemens N and P) over multiple days. The lower limit of detection (LLD) was determined as well as the reference interval (RI) using non-parametric analysis. Results: Results for 21 QC samples were obtained from seven individual calibration curves. No trending of results for either QC noted. The normal and abnormal QC had a coefficient of variation (CV) of 3.4% and 3.6%, respectively. The LLD was 0.04 U/mL (4%) which is similar to the manufacturers stated limit (3.5% for the ACL family). There was excellent correlation between the IL VWF:Ag and the Stago VWF:Ag assays (r²=0.97). The RI was 0.65 – 2.00 U/mL, which is similar to the manufacturer’s stated RI for A+B+AB blood groups (0.66–1.76 U/mL). Results from the patient samples tested spanned a wide range (0.02 – 0.92 U/mL) and showed excellent correlation (r²=0.93). Conclusions: Our results demonstrate excellent correlation between the IL VWF:Ag and Stago VWF:Ag assays performed on the STA-R. The RI and the LLD for the IL VWF:Ag is similar to that reported by the manufacturer. There was excellent correlation between patient samples. Our study illustrates a proof of principle: a LIA test can be run on a different manufacturer’s coagulation platform, with acceptable performance.

221 ACQUIRED HEMOPHILIA: A RETROSPECTIVE EVALUATION IN THE GHENT UNIVERSITY HOSPITAL
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Introduction: Acquired hemophilia A (AHA) is a rare bleeding disorder caused by autoantibodies against factor VIII. AHA occurs most frequently in elderly and in association with several conditions, such as autoimmune disease, pregnancy, malignancies, or drug exposure. However, in approximately 50 percent of the patients no underlying disorder can be identified. Methods: Retrospectively, we analyzed the characteristics and outcomes of the patients diagnosed with AHA in the Ghent University Hospital from January 2004 to November 2013. Results: Sixteen patients (seventeen episodes) were diagnosed with AHA, twelve were treated and followed in the Ghent University Hospital and four in a peripheral hospital. The median age of onset was 72 years and more than 75% of patients were 65 years or older comparable with data in literature. The male:female ratio was 9:8. We dispose of clinical data on twelve cases, they all presented with a bleeding event: bleedings into the skin, muscles, soft tissues or mucous membranes. At diagnosis the activated partial thromboplastin time (aPTT) was prolonged in twelve of the seventeen episodes with a median of 89 sec (no available aPTT measurements in 5 episodes). The median FVIII at diagnosis was 1% (0-49%), with most of the patients (77%) presenting with FVIII < 5%. The median inhibitor titer was 40 BU/mL, with a wide range (0.7-247 BU/mL, Bethesda assay). In 85% of the patients no underlying disorder could be identified. One case was associated with pregnancy. The basic therapeutic strategy involves control of the bleeding episodes with hemostatic agents and eradication of the autoantibodies by administration of immunosuppression. Recombinant activated FVII (NovoSeven®) was initiated in 54% of patients. Patients who reached complete remission (CR) - 58% - all received high dose corticosteroids. The inhibitor disappeared after a median duration of 52 days. One patient relapsed (7% vs 33.5% in literature) after 14 months of CR. We found a higher mortality rate (42%) compared to the literature (7.9%-22%), probably due to the transfer of critically ill patients to the Ghent University Hospital. Conclusions: The characteristics of
223 INTEREST OF THROMBIN TIME IN THE PERI-
PROCEDURAL MANAGEMENT FOR PATIENTS ON 
DABIGATRAN ETEXILATE

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CHU Dinant-Godinne UCL Namur, Namur Thrombosis and Hemostasis Center Yvoir; Belgium

Introduction: Possibilities to monitor the intensity of dabigatran etexilate (DE) treatment may be valuable before urgent intervention. Correlation between plasma dabigatran concentration and bleeding risk has been recently demonstrated. However, plasma levels where it is safe to carry out an invasive procedure or surgery have not been confirmed prospectively. The French Working Group on Perioperative Haemostasis proposed that the drug plasma concentration ([D]) should be less or equal to 30ng/mL. Consequently, there is a need for a rapid and widely available biological test. Some authors make proposals based on Thrombin Time (TT). However, TT is very difficult to standardize. Therefore, the objectives of this study were: - To determine the optimal [thrombin] with a variety of instruments/reagents - To assess the repeatability of TT at optimized conditions - To compare the sensitivity and linearity of TT at residual [dabigatran] (DA) with those of activated Partial Thromboplastin Time (aPTT) and Hemoclot Thrombin Inhibitor (HTI)

Methods: DA was spiked at increasing [] in pooled citrated normal human platelet-poor plasma (NPP). The following [DA] were prepared: 0, 5, 10, 20, 30, 40 and 50ng/mL. Bovine thrombin (HemosIL® TT) and human thrombin (STA®-Thrombin) were tested on 4 instruments: STA-R Evolution®, ACLTOP®, CS2000® and KC10®

Results: - The thrombin origin is a more important variable in comparison to the type of coagulometer. At [DA] of 30ng/mL and [thrombin] of 1.5 NIH/ml, the STA®-TT ranges from 56 sec to 74 sec according to the instrument, whereas on a same instrument, the TT varied of minimum 43 sec, depending on the thrombin origin. Except for STA®-Thrombin on STA-R®, the [optimized] is not the one recommended by the manufacturer (Table 1). Table 1 Optimized [thrombin] (NIH/ml) on 8 combinations reagent/instrument

<table>
<thead>
<tr>
<th>STA®-R®</th>
<th>ACLTOP</th>
<th>KC10®</th>
<th>CS2000®</th>
</tr>
</thead>
<tbody>
<tr>
<td>STA®-Thrombin</td>
<td>1.5</td>
<td>3.8</td>
<td>1.5</td>
</tr>
<tr>
<td>HemosIL® TT</td>
<td>3.8</td>
<td>5.0</td>
<td>5.0</td>
</tr>
</tbody>
</table>

- Repeatability experiments showed that variability increased with the [DA] (STA®-Thrombin (STA®-R®): coefficient of variation: 8.0% and 28.9% for 0ng/mL and 50ng/mL, respectively) and that the variability depends on the coagulometer and the reagent. - aPTT is not sensitive enough in low [DA], whatever the reagent, whereas HTI is not suitable in [DA] lower than 50ng/ml.

Conclusions: TT may be more informative than aPTT and HTI to provide with guidance to carry out an urgent procedure or surgery for patients receiving DE. However, TT is affected by a lot of analytic variables that should be understood by laboratories. Each laboratory should optimize its TT procedure according to its combination coagulometer-reagent.
from normal donors was spiked with various concentrations (range 75-600 ng in 1 mL of whole blood) of tissue-type plasminogen activator (tPA, Alteplase™, Genentech, CA, USA) and EXTEM was performed on ROTEM™ (TEM Systems Inc, NC, USA). Lysis onset time (LOT, time until clot firmness decreased by 15%) was recorded; tPA (normal range <10 ng/mL) and plasmin-antiplasmin complex (PAP) (normal range <750 µg/L) concentrations were measured in these samples using commercial ELISA assays. Data are presented as mean±SD. Results: The addition of tPA 75 ng was associated with increased fibrinolytic markers (5-6 folds from upper limit of normal range) but did not cause lysis pattern on ROTEM™ within 60 min. Hyperfibrinolytic pattern on EXTEM was traceable if tPA and PAP increased >10 times above normal range. It was calculated from exponential equation formula that tPA and PAP levels need average 81 ng and 6,250 mg/L, respectively, to show fibrinolysis pattern within 60 min run time of EXTEM.

<table>
<thead>
<tr>
<th>tPA added (ng)</th>
<th>LOT (min)</th>
<th>tPA (ng/mL)</th>
<th>PAP (µg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>75</td>
<td>&gt;60</td>
<td>67±22</td>
<td>3,630±2,050</td>
</tr>
<tr>
<td>150</td>
<td>37±5.9</td>
<td>128±26</td>
<td>8,950±2,110</td>
</tr>
<tr>
<td>300</td>
<td>17.0±1.7</td>
<td>272±57</td>
<td>16,970±6,240</td>
</tr>
<tr>
<td>600</td>
<td>6.7±0.2</td>
<td>505±41</td>
<td>31,360±10,870</td>
</tr>
</tbody>
</table>

Conclusions: Mild to moderate activation of fibrinolytic system is not sufficient to be detected by ROTEM™ within 60 minutes. Further studies are needed to evaluate if longer run of ROTEM™ (for 1.5-2 hours) would increase its sensitivity to mild or moderate activation of fibrinolysis, which is clinically significant for bleeding.

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**FLUORESCENCE MICROSCOPY AS BACKUP OF FLOWCYTOMETRY FOR DETERMINATION OF ACUTE LEUKEMIA LINEAGE IN LIMITED RESOURCES LABORATORY**

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**Introduction:** Normal 0 false false false EN-US X-NONE AR-SA

Multiparameter flowcytometry is the most powerful technology for immunophenotyping of acute leukemia. Immunophenotyping is crucial for the determination of therapeutic protocol. In developing countries with limited resource laboratories, sustainability of advanced technology such as flowcytometry is always an issue, especially with lack of technical support and backup equipment. Most of the times when a machine gets out of order the reagents are available but they got expired after a while. These situations may prohibit such laboratories from obtaining this essential technology. In attempt to put a solution for such situations, we evaluate the role of fluorescence microscopy in determination of acute leukemia lineage in emergency when flowcytometry is out of work. Methods: Fifty cases with initial diagnosis of acute leukemia, Sudan Black B negative and blast count more than 50% were included. Examination of wet preparation under binocular fluorescence microscope using 2 filters and the following antibodies: CD34, CD19, CD79a, CD10, CD3, CD5, CD117, CD13, CD33 &CD42. Antibodies are either labeled with FITC or PE. Flow check preparation was used with every batch. All those antibodies positivity in different types of acute leukemia have been checked previously in comparison with flowcytometry. Results: Fluorescence microscopy technique managed to determine the lineage in 68% of the cases. The best result was obtained in cases of B-ALL as 89.3% of suspected cases were confirmed. Six out of nine cases suspected to be T-ALL were proved. Most of the inconclusive results (76.9%) were obtained with suspected cases of AML (M0, M4, M5). The performance of antibodies have been evaluated. Conclusions: The methods probably gives the best estimate of the functional fibrinogen concentration in the patient. In the Maasstad Hospital, fibrinogen levels with a ‘fibrinogen curve error’ are now reported to the clinic, accompanied by a warning message.
FLOW CYTOMETRY IMMUNOPHENOTYPING OF 20 PATIENTS FROM BRAZILIAN PEDIATRIC MYELODISPLASTIC SYNDROME COOPERATIVE GROUP

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Introduction: Myelodysplastic syndrome (MDS) comprises a heterogeneous group of clonal hematopoietic stem cell diseases by ineffective hematopoiesis and subsequent frequent development of acute myeloid leukemia (AML). It is very uncommon in children, accounting for less than 5% of all hematopoietic neoplasms in patients less than 14 years old. Childhood MDS may be difficult to diagnosis, especially in cytopenic patients with normal or inconclusive morphology and normal cytogenetic, additional diagnostic markers are necessary. Flow cytometry (FC) has been used as an essential and important criterion for a better diagnosis, prognosis and therapeutic follow up in MDS. Here, we evaluate FC abnormalities in childhood MDS investigation and to verify the applicability of this technique in diagnosis. Methods: During eight months (May to December 2013), our group studied 20 bone marrow samples with suspected childhood MDS by 4-colors FC protocols (Cytomics FC500 and Navios, Beckman Coulter). We characterized the FC abnormalities, such as aberrancies in myeloid progenitor cells, granulocyte, erythrocyte and monocyte lineages, according to the European LeukemiaNet Working Group, 2012. The patients were followed up at Hospital Presidente Luiz Inácio Lula da Silva, Barretos, Brazil, and median age was 7 (1-16) years old. Results: In FC analyses, we consider significant abnormalities when more than three phenotypic alterations were found: 70% (14/20) of patients had changes in FC analysis and 30% (6/20) had no alterations. Among the abnormalities, the most common phenotypic alterations in neutrophil compartment were decrease in side scatter and maturation arrest, according to the score proposed by Ogata et al, 2010. In monocytic cells, we commonly found the expression of lineage infidelity markers such CD56. In erythroid population, we observed the abnormal relationship of CD36 and/or CD71 vs. CD235a. Among these patients, 10% (2/20) presented cytogenetic abnormalities (dell), 55% (11/20) did not achieve cytogenetic abnormalities, and 35% (7/20) did not have metaphases or request for study the karyotype. Conclusions: In our study we could evaluate the quantitative and qualitative changes in FC analyses in childhood patients with clinical hypothesis of MDS. Briefly, we found significant abnormalities related to the dysplasia in most of patients. However, considering that only few cases had karyotype alterations, FC could be used as an essential and important criterion when laboratory diagnosis is difficult to establish MDS in childhood.

EVALUATION OF ANTIGEN EXPRESSION INTENSITY IN THE DIAGNOSTIC OF CHRONIC LYMPHOCYTIC LEUKEMIA

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Introduction: B cell chronic lymphocytic leukemia (BCLL) is the most prevalent leukemic disease in the world characterized by the presence of at least 5000 clonal B lymphocytes in the peripheral blood which are CD5+, CD10-, CD19+, CD20-, CD22+, CD23+, CD79a, sigα+. The diagnosis is made by optical morphology study and flow cytometry analysis of lymphocytes to define the BCLL clone. In routine practice we just evaluate the expression or the lack of some antigens in B cells to identify the monoclonal phenotype of BCLL. Some authors postulated that the intensity of expression of the antigens presents in BCLL is different than normal lymphocytes B. In this study we evaluated the intensity of expression of B antigens in BCLL patients and compared them with normal B lymphocytes to attempt to characterize new criteria for the diagnosis of BCLL. Methods: The flow cytometric analysis was performed in an EPIX XL flow cytometer and we used the panel of diagnosis of BCLL including the following antibodies: CD19, CD10, CD20, CD22, FMC7, CD79b, CD45, IgM, CD5, CD103, CD25, CD11b and CD38. 40 patients with BCLL newly diagnosed and 30 healthy persons as controls were enrolled in this study. Student t test was used to compare the intensity of expression of CD19, CD20, CD22 and CD45 among the two groups by SPSS17 software. The intensity of expression was determined as the mean fluorescence ratio of B to T lymphocytes as a negative population to avoid handling fluctuations between samples. Regarding the CD45 expression we used the ratio of mean fluorescence of B lymphocytes to granulocytes. we considered a value of P< 0.05 as significant. Results: The expression of CD45, CD19, CD20 and CD22 were significantly lower in patients with BCLL than normal controls (P respectively 0.004, 0.029, 0.017, <0.0001). It is known that the expression of CD20 and CD22 is lower in BCLL was respectively 50% and 25% fewer than normal controls. Conclusions: The decrease of expression of some antigens especially CD45 and CD19 in BCLL can be helpful in the diagnosis of BCLL.
defined the normal differentiation patterns of various hematopoietic lineages. The aim of this work was to determine the usefulness of some phenotypic patterns used in our laboratory in the evaluation of low-grade MDS. We considered 41 low-grade MDS diagnosed between 2007 and 2013 at the Hematology Department of our Hospital: 20 Refractory Anemias, 3 Sg–Syndromes, 4 RARS, 14 RCMD. Methods: We analyzed the bone marrow of these patients in multiparameter FCM using the following combination of monoclonal antibodies up to 2011: CD11b/CD33/CD34/CD45/CD66b/CD117; CD10/CD11b/CD13/CD16/CD34/CD45; GlyA/CD36/CD45/CD71/CD105; CD14/CD33/CD36/CD45/CD56/CD64 (Becton Dickinson and Beckman Coulter). Since 2012 we have acquired two Navios Cytometers (Beckman Coulter), then we used the same antibodies in a ten color multiparametric FCM. For each sample analyzed in FCM we have acquired a minimum of 100,000 events. Results: The FCM analysis showed presence of several antigen expression aberrancies. The 65% of RCMD patients showed more than two anomalies. The same number of aberrancies was found in 50% of RA, 33% in Sq– Syndrome and 25% of RARS. The more frequent anomalies highlighted were low SSC, altered CD66b/CD11b and CD16/CD13 pattern on myeloid compartment and low CD10 expression in neutrophils. Conclusions: Morphology is not always clear-cut in the diagnosis of myelodysplastic syndromes (MDS). There is a need for additional diagnostic markers and FCM of bone marrow cells could be an important co-criterion in the diagnosis of MDS. In our low-grade MDS patient groups, FCM identify specific aberrations on both immature and maturing compartments among different hematopoietic lineages. These evidences could help the hematologists in MDS diagnosis.


Roy Edward, Ian Dimmick

INTRODUCTION: Reliable exclusion of dead/damaged cells is often needed for accurate flow cytometric evaluation of cell phenotypes. DRAQ7 is a far-red fluorescing cell viability probe; a water-soluble, DNA-binding, membrane impermeant anthraquinone dye, only labeling cells with compromised membranes (Akagi, 2013; Smith, 2013). Multi-colour analysis is limited by laser options, emission spectra and demand for bright fluors binding low concentration antigen densities on cells of interest. To minimize compensation issues within the higher quantum fluorescence dyes DRAQ7 is an ideal dead cell marker avoiding overlap with Brilliant Violet dyes, FITC or R-PE and minimal overlap with APC, and due to specificity/affinity, it provides clear s.n. DRAQ7 is optimally excited at 599/644 nm and, usefully, to blue wavelengths. In principle, multi-beam excitation means DRAQ7 occupies multiple channels, enabling selection of a unique population of dead cells, coincidently avoiding live cells; plotting independent far-red channels against each other with a laser and/or emission pairing that gives best separation of a double-positive population (dead/damaged cells) thus excluded from other channels. Conclusions: Anthraquinones have broad absorbance in the visible spectrum permitting detection in flow cytometers using blue to red excitation. Exploiting this, multi-beam excitation is demonstrated to discretely exclude dead/damaged cells (DRAQ7+) without channel cross-talk, utilizing the maximum number of channels. This is beneficial for the new violet-excited reagents.

311 EVALUATION OF THE BODY FLUID MODE ON THE XE-5000 FOR COUNTING LEUKOCYTES IN BRONCHOALVEOLAR LAVAGE FLUID

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1Department of clinical hematology, Agel laboratories Novy Jicin, Czech Republic, 2Department of immunology and serology, Agel laboratories Novy Jicin, Czech Republic, 3Department of pulmonary medicine, Hospital of Novy Jicin Novy Jicin, Czech Republic

Introduction: Bronchoalveolar lavage represents a widely applied diagnostic tool in pulmonary medicine. Standard methods of bronchoalveolar lavage fluid (BALF) analysis include flow cytometry and light microscopy. Routine hematology analyser XE-5000(Sysmex) offers the possibility to count body fluid cells using its extra body fluid mode. Several studies have been published recently examining the use of this mode for cell counting...
in body fluids such as cerebrospinal, synovial, peritoneal or pleural punctate. The aim of our study was to compare BALF analysis on hematology analyser with flow cytometry. The flow cytometry with specific antibodies is usually considered as the reference method. 

Methods: BALF samples of 120 pneumonology patients were analysed on XE-5000 (Sysmex) in body fluid mode and on flow cytometer (Cytomics FC 500, Beckman Coulter) simultaneously. The data obtained from hematology analyser relevant to our study involve total white blood cell (WBC) count and both total cell counts and ratios of polymorphonuclear cells, mononuclear cells and so-called “high fluorescent” body fluid cells (macrophages, mesothelial cells, malignant cells and other cells with high content of nucleic acid). Flow cytometer returned the differentiation of leukocytes in populations of lymphocytes, macrophages and polymorphonuclear cells. All samples were smeared, stained and checked by light microscopy. The correlation of the data received from flow cytometer and hematology analyser were evaluated by the Pearson correlation coefficient. 

Results: Total WBC counts of concentrated BALF’s varied from 0.055 to 41.494x10⁶ cells/l (mean 5,632, median 3,593 x10⁶ cells/l). The best correlation ratio returned analysis of mononuclear cells (lymphocytes, R=0.870, p<0.001). Numbers of high fluorescent body fluid cells and macrophages showed the correlation of R=0.686 (p<0.001). The correlation of polymorphonuclear cells counts was R=0.615 (p<0.001). Conclusions: BALF contain specific populations of leukocytes atypical in size, granularity and nucleic acid content. The main limitation of the routine hematology analyser is its automatic software gating of WBC populations causing inaccurate gating of abnormal cell types. To sum up routine hematology analyser can be effectively used for reliable total WBC count in BALF samples but differential leukocytes analysis lacks satisfying accuracy.

313 FLOWCYTOMETRIC IMMUNOPHENOTYPIC CHARACTERIZATION OF ACUTE MYELOID LEUKEMIA (AML) PATIENTS IN SUDAN
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Introduction: AML accounts for approximately 20% of acute leukemia in children and 80% of acute leukemia in adults. Immunophenotyping has become extremely important not only in diagnosis and subclassification of AML but also in the detection of the minimal residual disease. It is also suggested to have prognostic significance. The prognostic value of immunophenotyping in AML is controversial. This study was conducted for the first time in Sudan, to characterize acute myeloid leukemia presented to the flow cytometry unit at RICK (RadiolIsotope Centre Khartoum) which is the referral centre for cancer and the only centre running the service of immunophenotyping of acute leukemia in Sudan.

Methods: Case selection: 106 AML cases diagnosed at RICK by immunophenotyping were included in this study during the period mid 2010 and mid 2011. Flowcytometric immunophenotyping: bone marrow aspirate and peripheral blood samples from AML patients were collected and tested by Beckman-Coulter EPICS XL four color flowcytometer. Samples were stained by monoclonal antibodies for the following antigens: CD45, HLA-DR, CD34, CD117, CD13, CD33, CD19, CD7, cytoplasmic markers (CD3, CD79a, MPO), CD11c, CD14, CD64, CD42a, CD41 and CD61. Positivity was considered when ≥20% of the population expresses the marker. 

Results: Almost all AML blasts were expressing CD45 with the mean of 95.7% of them being positive with no differences between the subtypes. CD34 was positive in all the subtypes except for AML-M3 and AML-M3v (17.3% for both) and was positive in most population of AML-M0 (79.4%) and weakly positive in AML-M5 and AML-M7 (23.4% and 28.0% respectively), CD34 was studied in correlation to CD117 using Pearson correlation and found to have very strong positive correlation in AML-M3v, AML-M4 and AML-M7 (r=1.0, 0.928 and 0.962 respectively), and strong positive correlation in AML-M2 (r=0.763) while have strong negative correlation in AML-M3 (r=−0.689). CD13 and CD33 were also studied among the blast population having mean positivity of 51.5% and 49.8% respectively in all AML subtypes collectively. CD33 was found to have higher positivity among AML-M4 and AML-M5 with mean positivity of 75.9% and 76.6% respectively. CD13 and CD33 had no correlation for all AML subtypes except for AML-M5 with negative very strong correlation(r=−0.913). CD7 and CD19 was expressed in 54.9% 86.4% respectively. CD7 was mostly expressed in AML-M5 (76.9%) and least in AML-M3 while 100% of AML-M3v, AML-M4 and AML-M5 were positive for CD19 as aberrant expression. Conclusions: Flow cytometric analysis of AML is interpretive, combining the patterns and intensity of antigen expression to reach a definitive diagnosis. Some of the findings of this study were in agreement with similar studies while others were not.
samples - To validate imprecision and accuracy for the SOP, fresh whole blood anti-coagulated with K2-EDTA was collected from 50 healthy volunteers. For comparison study in nine laboratories, 65ml of fresh whole blood anti-coagulated with K2-EDTA was collected from 3 healthy volunteers. Aliquot volume(3.5ml) of whole blood samples were distributed to each laboratory for analysis. Measurement - Flowcytometric analysis was performed using FACS CantoII(BD Biosciences) in validation of the SOP and using FACS analyzers(BD Biosciences) and Navios(Beckman-Coulter) in comparison study at the nine laboratories. Statistics Analysis - Excel, StatisPro(CLSI) and MedCalc(Passing-Bablok regression, Bland-Altman plot) Results: The imprecision of lymphocytes, neutrophils, monocytes, eosinophils and basophils were CV% 0.5-1.1%, 0.3-0.8%, 0.9-2.1%, 1.1-2.2% and 2.8-3.4%, respectively. The results of each coefficient of correlation, slope and intercept were within the criteria of the CLSI H26-A2. Imprecision among 9 laboratories of lymphocytes, neutrophils, monocytes, eosinophils and basophils were CV% 2.4-5.0%, 1.6-2.5%, 4.0-9.5%, 3.5-8.7% and 3.1-12.5%, respectively. All results of 95% confidence intervals (95% CI) obtained by JSLH Diff were within the 95% CI obtained by the manual microscopic method. Conclusions: JSLH Diff including the SOP was validated as a reference method for leukocyte differential counts in blood for evaluation of automated hematology analyzers.

317 EARLY CHANGING PATTERNS OF T CELL CHIMERISM IN HAPLOIDENTICAL HEMATOPOIETIC STEM-CELL TRANSPLANTATION USING CD3-DEPLETED AND TCRαβ-DEPLETED GRAFTS

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Introduction: Haploidentical hematopoietic stem-cell transplantation (HHSCT) is an alternative transplant strategy for patients who lack a suitable HLA-matched donor. To determining the degree of engraftment in HHSCT, HLA antigens were used as markers to establish the presence of chimerism. Recently, several studies have been shown that HHSCT with TCRαβ-depleted graft is associated with good prognosis. In this study, we performed flow cytometry-based lineage-specific chimerism analysis to observe the early changing patterns of T cell population after two different strategy-based HHSCT: HHSCT using CD3-depleted graft (CD3-HHSCT) and TCRαβ-depleted graft (TCRαβ–HHSCT). Methods: Total eighteen pediatric patients were enrolled. Diagnoses were acute leukemia (n=10), aplastic anemia (n=6), non-Hodgkin’s lymphoma (n=1), and MDS (n=1). Patients were divided into 3 groups: CD3-HHSCT group, TCRαβ–HHSCT group, and HLA full-matched HSCT group (Full-HSCT group) as a reference. Flow cytometric analysis using anti-CD3, CD19, CD56, TCRαβ, and TCRγδ antibody were performed to identify T cell populations. We also selected a panel of specific antibodies to target HLA serotypes to be used in flow cytometric analysis, based on the allele distribution of donors and recipients. We then observed the changing patterns of T cell chimerism until post infusion date (PID) 7 and 14 in both CD3-HHSCT group and TCRαβ–HHSCT group. Additionally, we compared the counts of white blood cell (WBC), neutrophil, lymphocyte, TCRαβ, and TCRγδ among 3 groups at PID7 and 14. Results: The percentage of donor T cell began to increase earlier in TCRαβ–HHSCT group than CD3-HHSCT group, and reached to 90-100% at PID14 in both groups. The percentage of recipient T cell began to decrease earlier in TCRαβ–HHSCT group than CD3-HHSCT group, and reached to 0-10% at PID14 in both groups. TCRαβ counts were significantly lower in TCRαβ–HHSCT group at both PID7 (52.13% vs. 99.38%, P=0.006) and PID14 (60.33% vs. 98.38%, P=0.019). Similarly, TCRγδ counts were significantly higher in TCRαβ–HHSCT group at both PID7 (28.84% vs. 61%, P=0.006) and PID14 (18.19% vs. 61%, P=0.019). The counts of WBC, neutrophil and lymphocyte were not significantly different among three groups at PID7 and 14. Conclusions: In our study, early changing patterns of T cell chimerism showed difference between two different HHSCT groups. Therefore, monitoring the changing patterns of T cell chimerism seems to be more meaningful than monitoring of blood cell count. More studies of the dynamics of T cell reconstitution after different strategy of HHSCT is needed.

319 PROGNOSTIC VALUE OF FLOW CYTOMETRIC MEASUREMENT OF RESIDUAL DISEASE DURING THERAPY IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Russian Medicol Academy of Postgraduate Education Moscow, Russia

Introduction: The achievement of minimal residual disease - free status is associated with a better outcome in patients with chronic lymphocytic leukemia (CLL). The important direction of research is the identification of parameters useful for predicting response to therapy. The aim of investigation was to assess the prognostic impact of minimal residual disease (MRD) measured by flow cytometry in the peripheral blood of patients with CLL during therapy. Methods: The peripheral blood samples from 112 CLL patients (aged from 43 to 82 years) were analyzed using Cytomics FC500 (Beckman Coulter) and FACS CantoII (Becton & Dickinson) flow cytometers. All CLL patients received 6 courses of chemoimmunotherapies - the combination of fludarabine, cyclophosphamide, and rituximab (FCR). The MRD assessment was performed after 3 courses and after 6 courses using international standardized approach (Ravstron AC et. al. 2007). Cells were analyzed by 5- and 6-color flow cytometry for detection of CLL-associated immunophenotypes. Results: MRD-negative status was attained in 87 (78%) of patients after completion of therapy. Two patient risk groups were identified by MRD detected after 3 courses of FCR: 67 patients with low (<0.12% among leukocytes) and 45 patients with high (≥0.12% among leukocytes) leukemia cells levels. The frequency of identification of the MRD-free status after the end of treatment were 100% and 44% in these groups, respectively. Therefore, attainment of molecular remission after the end of treatment can be predicted when less than 0.12% leukemic cells are present among leukocytes after three courses FCR. Conclusions: The findings demonstrate a potential usefulness of the early immunophenotypic MRD assessment to predict differences in response to therapy for patients with CLL, that would be therefore useful to assign patients to different risk categories during first courses of treatment.
EFFECT OFERYTHROPOIETIN ONERYTHROID
PROGENITOR CELL OF ANEMIA IN CHRONIC DISEASE
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Introduction: Erythropoietin (EPO) is an important glycoprotein hormone in erythropoiesis. EPO induce the proliferation and maturation of erythroid progenitor cell. The purpose of this study is to investigate the effect of various concentration of EPO on cell proliferation, apoptosis and mRNA expression of erythroid progenitor cells treated with pro-inflammatory cytokine could be cause of anemia in chronic disease (ACD) patient compared with healthy subjects. Methods: CD34 positive cell were isolated from peripheral blood of healthy volunteer and ACD patient. The cells were cultured in 2 and 20 U/ml of EPO and treated cells with pro-inflammatory cytokines such as IL-1β and TNF-α for 7 days. Cell count, apoptosis and erythropoietin receptor mRNA expression were investigated by trypan blue, flow cyrometer by Annexin-V-FITC and Glycophorin A –PE staining and real time -PCR respectively. Results: The results showed that erythroid cells of healthy and ACD patient treated with EPO were increased with concentration dependent manner (Table 1.) The result of EPOR mRNA expression, 20U/ml EPO treated cells had higher EPOR mRNA expression than 2U/ml EPO at day 7 in all groups. Table 1. Total cell count and percentage of apoptosis of erythroid progenitor cell in various concentration of EPO and treated cell with 20ng/mL of IL-1β and TNF-α for 7 days (n=3)

<table>
<thead>
<tr>
<th>EPO</th>
<th>Cyto-</th>
<th>Cell count (x10^6 cells /μL)</th>
<th>Apoptosis (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>kinase (20ng/ml)</td>
<td>Healthy</td>
<td>ACD</td>
</tr>
<tr>
<td>2U/ml</td>
<td>IL-1β</td>
<td>226</td>
<td>154</td>
</tr>
<tr>
<td></td>
<td>TNF-α</td>
<td>213</td>
<td>184</td>
</tr>
<tr>
<td>20U/ml</td>
<td>IL-1β</td>
<td>278</td>
<td>211</td>
</tr>
<tr>
<td></td>
<td>TNF-α</td>
<td>291</td>
<td>248</td>
</tr>
</tbody>
</table>

Conclusions: EPO especially at concentration 20U/ml could induce cell proliferation, promote EPOR mRNA expression and inhibit apoptosis in erythroid progenitor cell of healthy and ACD patients. In addition, EPO could reduce apoptosis induced by pro-inflammatory cytokinetick therefore could be cause of anemia in chronic disease. This data could be use as information for EPO treatment in anemic patients particularly in anemia of chronic disease.

ASSESSMENT OF PLATELET FUNCTION BY A NEWLY DEVELOPED FLOW CYTOMETRY BASED ASSAY
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Introduction: Assessment of platelet function in whole blood by impedance aggregometry is increasingly used. However, it has recently been shown that this method is highly dependent on platelet count making it less suitable for examination of patients with thrombocytopenia. Aim To develop and validate a flow cytometry assay for assessment of platelet function by determination of the glycoproteins p-selectin, CD63 and bound fibrinogen expressed on the platelet surface. Methods: Background noise and spectral overlap were minimised by titration of the following antibodies with specific fluorochromes: CD42b-PE (R-Phycocerythrin), CD63-PC5 (Phycocerythrin-Cyanine 7), p-selectin-APC (Allophycocyanin), anti-fibrinogen-FITC (Fluorescein isothiocyanate) and isotype controls for PE and APC. Fluorescence minus one (FMO) controls were applied to validate the compensation matrix and the assay setup. The agonists arachidonic acid, ADP, collagen related peptide, ristocetin and Thrombin Receptor Activating Peptide-6 (TRAP-6) were titrated from maximum platelet response to no platelet response to find the optimal concentration with maximum platelet response for p-selectin, CD63 and bound fibrinogen. Platelet function was measured before and after stimulation with agonists in 20 healthy volunteers. Furthermore, platelet function was assessed in a patient with Bernard-Soulier with a low platelet count. Platelet function was defined as percentage of platelets positive for p-selectin, CD63 and bound fibrinogen. Results: The isotype controls showed minimal non-specific antibody binding, and the FMO controls showed no spillover-induced staining. The optimal added concentration of the agonists were found to be: 7.5 mM (arachidonic acid), 140 μM (ADP), 1.5 μg/ml (collagen related peptide), 130 μM (TRAP-6) and 12 mg/ml (low response) and 15 mg/ml (high response) for ristocetin. References intervals of the percentage of platelets positive for p-selectin, CD63 and bound fibrinogen were established from 20 healthy volunteers. Platelet function for the Bernard-Soulier patient was found to be normal when activated with arachidonic acid, ADP, collagen and TRAP-6, but missing when activated with ristocetin, which is in accordance with the diagnosis of Bernard-Soulier syndrome. Conclusions: We have developed and validated a flow cytometry assay for determination of platelet function. Future studies will validate the assay in patients with thrombocytopenia of different etiology.

THE DIFFERENCE BETWEEN THE NUMBERS OF LYMPHOCYTE EXPRESSING CO-STIMULATORY MOLECULES FROM HBV AND HIV-INFECTED INDIVIDUALS
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Introduction: The immune status might be affected by the co-stimulatory molecules expressing on T-cells. In persistent viral infection, the deteriorated immune system of HIV-infection is diverse between those in HIV infection. In this regard, the pattern of co-stimulatory molecules CD28 expressed on T-cells from HBV and HIV-infected individuals might be different. Therefore the aim of this study is to investigate the expression of co-stimulatory molecules on T-cells of HBV and HIV-infected individuals.

Methods: The total of 28 HIV and 25 CHB-infected patients whom were diagnosed and treated at Mahachakri-Srinindhorn Medical Center (MSMC) was recruited. HIV and HBV-infected subjects were similar with respect to gender and age. Whole blood was lysed and surface molecule staining CD3, CD4, CD8 and CD28 were performed according to standard protocol.

Results:
The means ratio of CD3 to CD3 expressing CD28 molecules in HBV and HIV-infected individuals were 1.5 and 1.7 respectively. For CD4 cells, it was found that mean ratio of CD4/CD4/CD28 in HBV and HIV-infection was 1.2 and 1.1 correspondingly. Considering to CD8, the mean ratio of CD8 to CD8 expressing CD28 molecule were 1.5 and 2.2 in HBV and HIV-infected patients. **Conclusions:** After normalization, the CD3+/CD28+ cells in patients with HBV infection were much more than those in HIV-infected individuals. The similar pattern, increasing in the number of CD8+/CD28+ was also found in the HBV infection. In contrast, for CD4, the CD4+/CD28+cells from HIV infection was much more than those in HBV infection. In addition, almost all CD4 positive cells showed CD28 molecules on their surface.

**327 RESULTS OF AN EXTERNAL QUALITY CONTROL CLL SAMPLE FOR FLOW CYTOMETRIC LEUKOCYTE DIFFERENTIATION BY CYTODIFF™ IN MULTIPLE LABORATORIES.**

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1Sint Franciscus Gasthuis Rotterdam, Netherlands, 2Bronovo Hospital Den Haag, Netherlands, 3Groene Hart Hospital Gouda, Netherlands, 4Zuwe Hofpoort Hospital Woerden, Netherlands, 5Sint Franciscus Gasthuis Assint, Netherlands, 6Nij Smellinghe Hospital Drachten, Netherlands, 7t Lange Land Hospital Zoetermeer, Netherlands, 8Isala Hospital Zwolle, Netherlands, 9Beckman Coulter Woerden, Netherlands

**Introduction:** Leukocyte differential counting can be performed by flow cytometry. Advantages over hemocytometry or microscopy are the increased counting statistics and the objective definition of leukocyte populations by immunologic markers. Cytodiff™(Beckman Coulter) is a CE-certified, 5-color, 6-antibody cocktail for a leukocyte differential by flow cytometry, combined with automatic gating software. It reports 17 leukocyte populations, including B- and T+NK-cells, different classes of blasts and progenitors (Faucher et al, Cytometry-A 2007, Roussel et al, Cytometry-A 2010). Several Dutch laboratories are either using Cytodiff™on their routine diagnostics, or are currently validating this technique. Participating laboratories tested the performance of Cytodiff™on a chronic lymphocytic leukemia (CLL) sample provided by SKML as “special sample”. **Methods:** Parallel to the regular external quality control (EQC) for hemocytometry issued by the Dutch Society for Quality Control in Medical Laboratories (SKML), a special CLL sample (2012-6-1) was provided to Dutch laboratories using Cytodiff™. The sample was sent fresh (unfixed) and participating laboratories were blinded to the diagnosis. The sample was measured using FC500 or Navios flow cytometers (Beckman Coulter). **Results:** This special sample was diagnosed in the send-out laboratory by means of traditional multicolor flow cytometry (FACSCanto II, BD-Biosciences). The cells were characterized as CD45pos, CD19weak, CD5pos, CD20pos, CD23weak, kappa-weak, lambda-weak and CD10neg. The diagnosis was monoclonal B-cell population, compatible with a B-CLL. All participating laboratories identified B-cells as aberrant cells by Cytodiff™(CD19weak). The B-CLL cells were classified by the auto-gating software as all B-cells in 3 laboratories (88-91% B-cells), or as a combination of B-cells (58-63%) with blasts (21-30% Xn) in 2 laboratories, or as B-cells (56%) combined with T-cells (37%) in 1 laboratory. In figure 1, some of the cell populations that are measured using Cytodiff™are on the X-axis, the percentage of cells is on the Y-axis. The results for the B-CLL sample for the 6 different laboratories are given in different colors. **Conclusions:** External quality control of fresh pathological blood samples provides a valuable tool for robust implementation of Cytodiff™in the medical laboratory. All laboratories detected aberrant B-cells (CD19weak) in the CLL sample, but the classification of these cells by the automatic gating software differed between laboratories. Comparing results between participating laboratories provides a valuable platform for discussing analytical, logistical and reporting aspects.

![Figure 1: Cytodiff results of an EQC-B-CLL sample in 6 laboratories](image)

**401 TELOMERASE REVERSE TRANSCRIPTASE (TERT) A1062T MUTATION AS A PROGNOSTIC FACTOR IN EGYPTIAN PATIENTS WITH ACUTE MYELOID LEUKEMIA (AML)**

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Mansoura University Mansoura, Egypt

Objective: Loss-of-function mutations in telomerase complex genes reduce telomerase activity, which predisposes to acute myeloid leukemia (AML). This study aimed to evaluate the incidence and prognostic impact of the most common TERT mutation A1062T in AML patients treated at Mansoura Oncology Center. **Methods:** Mutation screening for TERT (A1062T) mutation on exon 15 of the TERT gene was performed on diagnostic DNA samples from 153 AML patients, by using sequence specific primers. TERT (A1062T) mutation was detected in 18 cases out of 153 patients (11.8 %). **Results:** There was no difference between the two groups as regard sex, French-American-British subtypes, cytogenetics status (favorable or intermediate/adverse) and presenting blood counts. On the other hand, there were statistical differences in age, type of leukemia (de novo or secondary), extramedullary invasion and performance status. The induction remission rate (CR) was significantly higher in AML patients without TERT
(A1062T) as compared to mutated AML patients (53.3% vs 16.7% respectively). Moreover, the relapse rate was significantly higher in AML patients with mutation as compared to those with wild type (62.5% vs 28.2% respectively). As regard the overall survival (OS), the patients with TERT (A1062T) mutations had shorter overall survival (OS) than patients with wild type (P = 0.023). In a multivariable analysis, TERT (A1062T) mutational status is independently worse predictor factor (P = 0.036) when controlling for cytogenetic status (P = <0.001), Denovo or secondary AML (P = 0.003) and bone marrow blast cells (P = 0.042). Discussion: In conclusion, TERT A1062T mutation is an independent negative prognostic factor in AML patients. Therefore, molecular testing for TERT A1062T mutation in patients with AML is recommended in order to delineate their prognostic status.

403 AUTOMATED QUANTITATIVE BCR-ABL ASSAY USING GENEEXPERT : A TWO YEAR FOLLOW UP OF VALIDITY AND STABILITY OF CONVERSION FACTOR
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Introduction: The BCR-ABL fusion protein characterises myeloproliferative neoplasm chronic myeloid leukaemia (CML). Quantitative monitoring for bcr-abl traditionally uses reverse transcriptase polymerase chain reaction (qPCR); is considered critical in monitoring and change of therapy paradigms for CML. More recently, an automated cartridge based detection system (GeneXpert,Cepheid) has become available. Methods: A retrospective analysis of results obtained by GeneExpert and by qPCR from 08/2010 to 06/2013 has been compared including 6 monthly validation cycles.129 peripheral blood samples were selected on the basis of the detectable value in MMR range (~0.1% IS).Bland and Altman method and bias plots were used for comparison. Results: The initial correction factor was 0.43. The results of verification cycles are in Table 1. All results except second cycle were within desirable limits (Table 2) which resulted in change of conversion factor to 0.47. The Bland-Altman plot for all cycles shows good agreement except for 5 results which were considered outliers. Table 1.Concordance between the GeneExpert in HAPS and reference method IMVS Adelaide

<table>
<thead>
<tr>
<th>Cycle</th>
<th>Bias (CI)</th>
<th>Lower limit of agreement</th>
<th>Upper limit of agreement</th>
<th>Antilog of Bias</th>
<th>Mean bias from ref method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline validation</td>
<td>0 (-0.171 to 0.171)</td>
<td>-0.736 (-1.011 to -0.462)</td>
<td>0.736 (0.462 to 0.111)</td>
<td>1.00</td>
<td>0.00</td>
</tr>
<tr>
<td>1st cycle</td>
<td>-0.078 (-0.239 to 0.082)</td>
<td>-0.840 (-1.100 to -0.579)</td>
<td>0.684 (0.423 to 0.944)</td>
<td>0.84</td>
<td>-1.2</td>
</tr>
<tr>
<td>2nd cycle</td>
<td>-0.273 (-0.384 to -0.162)</td>
<td>-0.764 (-0.943 to -0.585)</td>
<td>0.217 (0.038 to 0.396)</td>
<td>0.53</td>
<td>-1.9</td>
</tr>
<tr>
<td>3rd cycle</td>
<td>-0.012 (-0.172 to 0.148)</td>
<td>-0.506 (-0.749 to -0.262)</td>
<td>0.481 (0.283 to 0.725)</td>
<td>0.97</td>
<td>-1.0</td>
</tr>
<tr>
<td>4th cycle</td>
<td>-0.028 (-0.097 to -0.154)</td>
<td>-0.620 (-0.826 to -0.414)</td>
<td>0.677 (0.471 to 0.883)</td>
<td>1.07</td>
<td>-1.1</td>
</tr>
</tbody>
</table>

Conclusions: Establishing method specific correction factor and ongoing precision and accuracy requires regular assessment. We observed a lot to lot variation as well a change in correction factor over time. The resultant change in correction factor reverted the bias to more acceptable range as seen in third and fourth verifications. Regular verification with a reference laboratory is useful to monitor drift in IS measurements for automated instruments.

405 DYSREGULATION OF MIR-124-1 PREDICTS FAVORABLE PROGNOSIS IN ACUTE MYELOID LEUKEMIA
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Introduction: MicroRNA miR-124 has been suggested as a tumor suppressor for its role in inhibiting cell growth, inducing differentiation and promoting apoptosis. The present study was aimed to investigate the expression status of miR-124-1 and its
407 DETECTION OF T315I MUTATION OF BCR-ABL GENE IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA BY ALLELE-SPECIFIC PCR.

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Introduction: The T315I mutation in the BCR-ABL gene causes the development of resistance to all tyrosine kinase inhibitors currently used in clinical practice as a target therapy for chronic myeloid leukemia. The method of Sanger sequencing allows to identify all the possible types of mutations in the kinase domain, but its low sensitivity can not detect the mutant clone, if it does not exceed 20% BCR-ABL positive cells.

Methods: We analyzed 46 samples of peripheral blood from patients with chronic myeloid leukemia. As a control, we used BCR-ABL positive K562 cell line, which has no mutation in the kinase domain, and the 10 samples of peripheral blood of patients with BCR-ABL negative patients samples. In all samples was determined the relative expression of the BCR-ABL gene. In BCR-ABL positive samples we determined the kinase domain mutations of BCR-ABL by direct sequencing. PCR for detecting BCR-ABL point mutation T315I and calculation of mutant transcripts level was carried out in two stages. At the first stage of the chimeric transcript was amplified, then Allele-specific (AS)PCR for detecting ABL T315I point mutation was second stage was performed. Results: The T315I mutation (AS-PCR) was detected in all samples in which the presence of the mutation was identified by direct sequencing method (n = 10) and was not detected in any of control samples obtained from patients with BCR-ABL- negative lymphoid malignancies (n = 10) and the cell line K-562. Sensitivity of the method was determined by serial dilutions, obtained by mixing cDNA samples obtained from patients with the mutation T315I, and the cell line K-562. Positive control samples and dilutions of 1:10 and 1:100 were positive for T315I by AS-PCR in 100% cases, as a result of 1:500 dilution - in 80% cases, 1:1000 dilution - in 75% cases. Retrospective analysis of the samples from the T315I-positive patients, showed that the AS-PCR reveals the emergence of a mutant clone in 6 months before detection by direct sequencing, but mutation was not detected in samples obtained at 12 and 9 months up to this point (Figure 1).

Conclusions: Compared direct sequencing and allele specific PCR enables to perform the detection of small amounts (1% or less) transcripts with the presence of a T315I mutation among the wild-type transcripts. Identifying of patients with T315I mutations in a small number of transcripts would have more time to search for an unrelated donor, and preparation for transplantation.

409 EXOSOMES DERIVED FROM K562/G01 CELLS REDUCE APOPTOSIS OF K562 CELLS TREATED WITH IMATINIB

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Introduction: Chronic myeloid leukemia (CML) is a clonal hematopoietic stem cell disorder. Imatinib has become the standard first-line therapy for CML. However, as clinical experience with imatinib grows, it is clear that some patients are resistant to imatinib, and cytogenetic response does not occur, the mechanism of imatinib resistance are not fully elucidated. Up to now, there were many researches demonstrated that leukemia niche plays an important role in imatinib resistance. Further, exosomes are important element of tumor microenvironment, and it can deliver miRNA to recipient cells and influence the functions of recipient cells. We hypothesized that miRNAs are contained in exosomes in K562 and K562/G01 cells and there exists difference of miRNA expression in exosomes from them, the miRNAs in exosomes from K562/G01 cells may related with the effect of Imatinib resistance, can influence the function of K562 cells.

Methods: We performed miRNA microarray on K562 exosomes and K562/G01 exosomes by using the 6th generation of miRCURY™ LNA Array, and confirm the results of miRNA expression from microarray by using fluorescence quantitative PCR. We also detect the apoptosis of K562 cells cocultured with exosomes from K562/G01 by using flow cytometry, when treated with imatinib.

Results: We found that 21 miRNAs were up-regulated and 29 miRNAs were down-regulated in K562/G01 exosomes. And then we conduct Gene ontology analysis and Kyoto Encyclopedia of Genes and Genomes(KEGG) analysis for these selectively secreted miRNAs. The GO terms and signaling pathways associated with targets were categorized into 63 classes, including stem cell maintenance, cell adhesion, which are related to imatinib resistance. And 48 signaling pathways(such as pathways in cancer, focal adhesion and chronic myeloid leukemia), exosomes derived from K562/G01 cells can reduce apoptosis of K562 cells treated with Imatinib.

Conclusions: As we know, our study
is the first time to compare the miRNAs expression difference between K562 exosomes and K562/G01 exosomes and analyze the selectively encapsulated miRNAs’ function, we demonstrated that exosomes derived from K562/G01 cells can reduce the apoptosis of K562 cells treated with Imatinib, it may provide a new mechanism of understanding imatinib resistance from a new angle.

411 DIFFERENTIAL EXPRESSION AND ALTERNATIVE SPlicing OF CELL CYCLE GENES IN IMATINIB- TREATED K562 CELLS
liu jing, huang bo, chen xin, xu yanmei, chen ximin, wang xiaozhong
Nanchang University Nanchang, China

Introduction: Cancers are diseases where regulation of the cell cycle is defective. There are a number of chemotherapy drugs that have shown to have the abilities to induce cell cycle arrest and to play an important role in cancer therapy. The purpose of the present study was to comprehensively investigate the effects of imatinib on the expression profiling of cell cycle genes in human myeloid leukemia cells K562. In addition, as the importance of relationship between RNA splicing and cell cycle progression, we also investigated the alternative splicing of the cell cycle genes that affected by imatinib. Methods: K562 cells were grown in RPMI 1640 media supplemented with 10% fetal bovine serum in a 5% CO2 humidified atmosphere at 37°C. Human Exon 1.0 ST Array analysis was performed using total RNAs purified from control and imatinib-treated K562 cell groups. Data from the exon arrays were processed using easyExon software. RT-PCR was used to confirm the differentially expressed and alternatively spliced genes. Results: A total of 2193 transcripts were considered significantly differently expressed in control and imatinib-treated K562 cells. From the differentially expressed total transcripts, we identified 185 differentially expressed genes and 277 alternative splicing events between the two cell groups. We list several differentially expressed genes and 277 alternative splicing events in Table 2.

Table 2. Alternative splice probesets of cell cycle-related genes that were significantly changed in K562 cells in response to imatinib treatment

<table>
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<th>Gene probe ID</th>
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<td>3145124</td>
<td>8.14</td>
<td>CCNE2</td>
<td>NM_057749</td>
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</table>

Conclusions: This information may help improve the mechanism of imatinib therapy in patients with chronic myeloid leukemia (CML) and may be useful for imatinib resistance research and CML drug development.

413 GENE MUTATIONS IN ACUTE MYELOID LEUKEMIA
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Introduction: Acute myeloid leukemia (AML) is a clinically, morphologically and genetically heterogeneous disease, involving one or all myeloid lineages. The heterogeneity of AML is reflected by differences in molecular abnormalities that have been recently discovered as potential prognostic factors in acute myeloid leukemia (AML). A genetic-based classification is essential for accurate diagnosis, prognostic stratification, monitoring of minimal residual disease and developing targeted therapies. Consequently, it is important that any screening panel is large enough to detect relatively rare mutations that might associate with a particular AML class or cytogenetic risk group. Methods: A variety of AML-specific mutations were screened, i.e. FLT3, NPM1, IDH1, IDH2, CEPBA, DNMT3A, RUNX-, KIT, WT1, IDH1, IDH2, and PML-RARA. In bone marrow samples of 66 adult patients, AML Patients using Sequencing technology during the period of 2012 to 2013 at the Molecular Genetics Laboratory, King Faisal Specialist Hospital and Research Centre, Riyadh (General organization).

Results: A total of 66 patients (28 females and 38 males) were investigated for different gene mutations (56 cases at the time of diagnosis, 4 patients in relapse, 5 samples for patients prior to stem cell transplantation and one case post bone marrow transplantation in relapse). The positive case represented 51% (34 cases of AML/28 adults and 6 pediatric patients). Conclusions: The frequency of different gene mutation in AML was similar to previously published studies from different international centers (e.g. FLT3-ITD and NPM1 are 20% and 23% respectively), however, this is the first data presented from Saudi Arabia.
415 KILLER IMMUNOGLOBULIN-LIKE RECEPTOR GENE TYPE IN KOREAN PATIENTS WITH APLASTIC ANEMIA
Bo Hyun Kim1, Seongsoo Jang2, Yu-Jin Lee2, Nure Park2, Youngjin Kim1, Sang-Hyuk Park1, Young-Uk Cho1, Chan-Jeoung Park1, Eun-Seok Choi2, Kyung-Nam Koh1, Ho-Joon Im3, Jong-Jin Seo1, Hyun-Sook Chi1
1Department of Laboratory Medicine, University of Ulsan College of Medicine and Asan Medical Center Seoul, Korea, 2Asan Clinical Research Center, University of Ulsan College of Medicine and Asan Medical Center Seoul, Korea, 3Department of Pediatrics, University of Ulsan College of Medicine and Asan Medical Center Seoul, Korea

Introduction: Human killer immunoglobulin-like receptor (KIR) of human NK cells are expected to play important role in the pathophysiology for bone marrow failure syndrome and successful allogenic stem cell transplantation. In aplastic anemia (AA), tendency of reduced frequency of certain KIR genes like 2DS1 or 2DS5 in patient cohort was reported. Study on KIR gene frequencies was done in Korean AA pediatric patients. Methods: KIR genotyping was performed on DNA from peripheral blood (PB) specimens of 11 pediatric patients with AA. Genomic DNA was extracted using the QIAamp DNA blood mini kit (Qiagen, Hilden, Germany). PCR was performed using KIR Genotyping SSP Kit (Invitrogen-LifeTechnologies, Inc., Carlsbad, CA) following the manufacturer's protocol. Frequency of KIR genes were compared with Korean population data from 154 individuals. Results: Eight males and three females were included. The median age (range) of patients included was 13 (3-17). KIR genes showed the frequency: 2DL1 (100%, 11/11), 2DL2 (0%, 0/11), 2DL3 (100%, 11/11), 2DL4 (100%, 11/11), 2DL5 (64%, 7/11), 2DS1 (64%, 7/11), 2DS2 (0%, 0/11), 2DS3 (36%, 4/11), 2DS4 (91%, 10/11), 2DS5 (36%, 4/11), 3DL1 (100%, 11/11), 3DL2 (100%, 11/11), 3DL3 (100%, 11/11), 3DS1 (45%, 5/11), 2DP1 (100%, 11/11) and 2DP (100%, 11/11) and 3DP1 (100%, 11/11). Among them, frequency of 2DS1 (65%, 7/11) and 2DS5 (36%, 4/11) of patients with AA did not showed tendency of reduced frequency relative to healthy controls from data of Korean population (37.7% and 26.6%, respectively). However, 2DS2 with 16.9% of frequency in Korean population was not found in our study group (0%, 0/11). Conclusions: The KIR2DS2 was not observed in our study group. The frequency of KIR gene in AA is required to be studied by ethnic group.

417 CIRCULATING CRYOglobulINS AND GLOMERULONEPHRITIS: A RETROSPECTIVE ANALYSIS OF 187 CASES OF PATIENTS
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Introduction: Previous studies on cryoglobulinemic glomerulopathy were based upon case series that were performed before hepatitis C virus infection was investigated, and there have only been a few reports about HCV-negative cryoglobulinemic glomerulopathy. Through analysis of the blood, urine and kidney biopsy histopathology data received from glomerulonephritis patients that had cryoglobulin and hepatitis C virus-testing, to observe the specific histological features and blood, urine indicators of HCV-negative cryoglobulinemic glomerulopathy patients, and to explore the role of primary cryoglobulinaemia in the pathogenesis of glomerulonephritis. Methods: 187 patients with glomerulonephritis diagnosed by renal biopsy who underwent examination for Circulating cryoglobulins and HCV infection at Peking University First Hospital between 2008 and 2012 were recruited for this study. Patients with complications such as underlying lymphoproliferative disorders, autoimmune diseases, infection, and liver disease were excluded. Patients were divided into groups according to the histopathological type and presence or absence of circulating cryoglobulins. Results: In the membranous nephropathy group (n = 45), 8 patients were cryo-positive (17.8%). In the crescentic glomerulonephritis group (n = 32), 6 patients were cryo-positive (18.8%). IgA nephropathy (n = 43) including proliferative IgA nephropathy (n = 29) out of which 3 cases (8.3%) were cryo-positive, sclerosis IgA nephropathy (n = 7) with no cryo-positive cases. In the atypical membranous nephropathy group (n = 13), 5 patients were cryo-positive (38.5%). In the mesangial proliferative glomerulonephritis (n = 22), 7 patients were cryo-positive (31.8%). In the minor glomerular abnormalities group (n = 19), 4 patients were cryo-positive (21.1%). In the focal proliferative glomerulonephritis (n = 7), 5 patients were cryo-positive (71.4%). One out of the two endocapillary proliferative glomerulonephritis cases was cryo-positive. No cryo-positive cases were found in the 4 focal Segmental Glomerulosclerosis cases. Compared with the cryo-negative group, the cryo-positive group had lower serum immunoglobulin IgG levels in membranous glomerulonephritis patients (p<0.05). In atypical membranous glomerulonephritis patients, cryo-positive group had predominant IgM staining. In patients with membranoproliferative glomerulonephritis, the cryo-positive group had higher 24-hour urinary protein, lower serum total protein and albumin levels, higher serum immunoglobulin IgM levels, predominant IgM staining. Unlike the predominant staining for IgM in the cryo-positive group, Patients with cryo-negative membranoproliferative glomerulonephritis showed predominant staining for IgG. Conclusions: A high proportion of almost all histological types of glomerulonephritis are found cryo-positive, especially membranoproliferative glomerulonephritis, focal proliferative glomerulonephritis and atypical membranous nephropathy. Cryo-positive glomerulonephritis shows a close relationship with IgM. Cryo-negative membranoproliferative glomerulonephritis patients have more severe hypoalbuminemia, and more proteinuria than cryo-negative cases. Cryo-negative membranoproliferative glomerulonephritis has a close relationship with IgG staining.

419 MATERNAL NON-INVASIVE FETAL DNA TEST USED IN PRENATAL DIAGNOSIS
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Introduction: Down’s syndrome is a frequently seen disease among newborns. Incident rate is about 1 per 750 newborns. There are about 26600 Down’s newborns each year and 1 million patients in China. To evaluate the diagnostic accuracy and sensitivity of non-invasive fetal trisomy test (NIFTY). NIFTY is newly developed test to detect fetal DNA in maternal blood for prenatal screen ofeatal autosomal and sex chromosomal aneuploidies. Methods: NIFTY which weredone by GBI lab China, utilizes EDTA anticoagulated blood from mother. This test is to detect fetal chromosomal abnormalities in the maternal samples with new generation high-throughput technologies, combined with advanced bioinformatic analysis. NIFTY test can provide an estimation report of the fetal risk being affected by Trisomy 13, 18 and 21, and also can give remind on abnormal sex chromosome. Results: Total 218 patients detected in 2012, 8 positive results were 3 Trisomy 21, 2 Trisomy...
18, 1 Trisomy 13 and 2 sexual chromosome X0, other 210 results were normal and 90 of them have delivered normal babies by end of 2012. The positive rate was 3.67%. All positive cases have been confirmed by cytogenetic tests. No abnormal babies were found in normal result cases so far. So test specificity was 100%, no false negative case was found so far. **Conclusions:** NIFTY is a very good test to detect fetal chromosomal aneuploidies. There is absolutely no risk of miscarriage or harm to fetus. Comparing to the first and second trimester maternal serology screen tests, fetal DNA tests are more reliable, more sensitive with high accuracy. Comparing with amniocentesis cytogenetic tests, NIFTY is more safe, no damage to fetus. Any pregnant woman who wants prenatal screening or diagnosis of fetal Down syndrome may consider adopting the NIFTY test. Pregnant women who already have a positive traditional blood screening test for Down syndrome may consider the NIFTY test to avoid unnecessary invasive test due to false positivity. The NIFTY test should be offered as a screening test for fetal Down syndrome to pregnant women with a singleton pregnancy at 12 weeks of gestation or beyond.

421 VALIDATION OF A REAL-TIME PCR ASSAY TO DETECT KIT D816V MUTATION IN SYSTEMIC MASTOCYTOSIS
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**Introduction:** An activating point mutation in the KIT gene resulting in an amino acid change from aspartic acid to valine at position 816 (D816V) is prevalent in >95% of cases of systemic mastocytosis (SM). Identification of this mutation in bone marrow (BM) or peripheral blood (PB) can assist in the diagnosis of SM and in the evaluation of atypical mast cell proliferations seen in a variety of reactive conditions. We have analytically validated a real-time PCR assay using Amplification Refractory Mutation System (ARMS) and compared it to Competitive Allele-Specific TaqMan PCR (castPCR) for accuracy and sensitivity. **Methods:** For both ARMS (Qiagen) and castPCR (Life Technologies), two separate reactions were performed for each specimen to determine cycle threshold (Ct) for gene reference assay (non-variable region of target gene) and mutation allele assay. Reference range Ct was established for gene reference assay using genomic DNA from D816V negative specimens (PB, n=100; BM, n=20) and for mutation assay using a D816V mutant cell line DNA. Specimen yielding a delta Ct (Mutant Ct- Reference Ct) corresponding to 0.1% mutant allele was considered positive. DNA from PB with yielding a delta Ct (Mutant Ct- Reference Ct) corresponding to 0.1% mutant allele was considered positive. DNA from PB with high Hb F determinants, including δβ-thalassemia and hereditary persistence of fetal hemoglobin (HPFH), are associated with diverse molecular and phenotypic features. We have examined in a large cohort of these conditions in Thai population. **Methods:** Study was done on a cohort of 160 adult subjects with differences in Hb F expression. Hematologic parameters and Hb analysis were recorded. DNA deletions causing δβ-thalassemia and HPFH were identified using multiplex gap PCR and denaturing HPLC assays. **Results:** DNA analysis identified the 12.6 kb deletion δβ-thalassemia in 79 cases (49.4%), the 7.2 kb deletion HPFH1-6 in 65 cases (40.6%), the Indian deletion inversion γ(γδβ)-thalassemia in 15 cases (9.4%) and a Chinese δβ(γδβ)-thalassemia in 1 case (0.6%). Eighteen (11.3%) cases were found to carry α-thalassemia with 10 different genotypes. Differences in hematological features as well as Hb F levels were noted. **Conclusions:** The hematological findings indicate that it is very hard to differentiate these high Hb F conditions at routine hematologic analysis. Comparison of phenotypes, genotypes and the deletion breakpoints indicates that differences in Hb F expression are correlated with the existence of α-thalassemia, the loss of BCL11A binding region located 5′to the δ-globin gene and the 3′β-globin enhancer which confirms their important roles in fetal Hb expression.

425 OVEREXPRESSION OF MIR-378 IS FREQUENT AND MAY AFFECT TREATMENT OUTCOMES IN PATIENTS WITH ACUTE MYELOID LEUKEMIA
Jun Qian1, Jiang Lin1, Wei Qian1, Ji-chun Ma1, Si-xuan Qian1, Yun Li1, Jing Yang1, Jian-yong Li1, Cui-zhu Wang1, Hai-yan Chai1, Xing-xing Chen1, Zhao-qun Deng1
1Affiliated People’s Hospital of Jiangsu Zhenjiang, China; 2The First Affiliated Hospital of Nanjing Medical University Nanjing, China

**Introduction:** MicroRNA miR-378 plays important roles in tumorigenesis by enhancing cell survival, reducing apoptosis, promoting tumor growth, angiogenesis and promoting cell migration and invasion. Abnormal expression of miR-378 has been observed in various types of cancers. However, the pattern of miR-378 expression remains unknown in acute myeloid leukemia (AML). **Methods:** The expression status of miR-378 was analyzed in AML using real-time quantitative PCR. **Results:** miR-378 overexpression versa due to minor difference in delta Ct cut-off (∓ ~0.5 Ct). ARMS had a high precision of Ct for both reference and mutant assays (CV <2%), and it was robust with a range of input DNA (PB, 10-40 ng; BM, 25-50 ng). **Conclusions:** ARMS, chosen for logistics, is an accurate and sensitive method to detect KIT D816V mutation in the diagnosis of Systemic Mastocytosis.
was identified in 26 of 84 (31%) AML patients. The patients with miR-378 overexpression had lower hemoglobin level than those without miR-378 overexpression (66 versus 75 g/l, respectively, *P*=0.010). The frequency of miR-378 overexpression in FAB-M2 subtype was higher than other subtypes (44% versus 20%, *P*=0.032). Moreover, the frequency of miR-378 overexpression was higher in patients with t(8;21) than in others (64% versus 24%, *P*=0.012). The status of miR-378 expression was not correlated with the mutations of eight genes (FLT3-ITD, NPM1, C-KIT, IDH1/IDH2, DNMT3A, C/EBPA and U2AF1). The difference in relapse-free survival was observed between patients with and without miR-378 overexpression (*P*=0.049). **Conclusions:** miR-378 upregulation is a common event and might have an adverse impact on prognosis in AML.

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**NEXT GENERATION SEQUENCING FOR GENETIC DIAGNOSIS OF HAEMOGLOBINOPATHIES**

Claire Shooter1, Swee Lay Thein2, Barnaby Clark2

1King’s College London London, United Kingdom; 2King’s College Hospital London, United Kingdom

**Introduction:** Haemoglobinopathies are caused by a wide range of mutations, ranging from single base changes to large rearrangements of over a megabase. Given the repertoire of mutations, diagnostic labs often resort to several different techniques to identify the causative mutations in cases they receive. Next Generation Sequencing (NGS) platforms are becoming increasingly available in diagnostic laboratories. Many studies have found that when teamed with the correct analytical software, NGS provides exceptional accuracy in mutation detection: low copy mutations, large (whole exome) target regions and numerous samples can be successfully analysed in parallel. This study evaluates NGS as a tool for DNA diagnosis of the haemoglobinopathies. **Methods:** Four DNA samples from patients with large rearrangements affecting the beta globin gene locus were selected for NGS: two samples had known deletions listed in the HbVar database, two samples had uncharacterised mutations - one a suspected duplication and the other a suspected deletion. A normal control sample with no rearrangements was also selected. DNA was prepared for sequencing with the SureSelect Library Preparation Kit (Agilent). A SureSelect Custom Target Enrichment Kit (Agilent) was used to enrich for targeted sequence from a 4Mb region of chromosome 11 encompassing the beta globin gene cluster. Samples were pooled and sequenced on a single flow cell on the MiSeq platform (Illumina). **Results:** Sequencing data was quality filtered and aligned to a reference sequence using NextGene software (SoftGenetics). By comparing the number of reads aligned to the reference between the samples and the normal control, it was possible to identify deleted or duplicated regions. All four rearrangements were identified in this way with a maximum resolution of +/-120bp. Reads which crossed the rearrangement break-points could not be correctly aligned to the reference sequence. These break-point reads were identified by their partial alignment to the start and end points of the rearrangements. Identification of these reads provided to-the-base resolution of both the known and unknown rearrangements (including a 50kb inversion). The findings for the unknown rearrangements were confirmed by Gap-PCR. Characterisation of the unknown duplication is illustrated in Figure 1. **Conclusions:** NGS Sequencing can be used to characterize large duplications, deletions and down to single base changes. Further work is required to develop a reliable analysis protocol as break-point detection was not straightforward in every case.

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**DOES THE TYPE OF BCR-ABL1 FUSION TRANSCRIPT REALLY IMPACT ON THE RESPONSE IN PEDIATRIC PATIENTS WITH CHRONIC MYELOID LEUKEMIA?**

Anita Tahlan, Neelam Varma, Shano Naseem, Jogeshwar Binota, Pankaj Malhotra, Deepak Bansal, Subhash Varma

Postgraduate Institute of Medical Education & Research Chandigarh, India

**Introduction:** Over the years remarkable progress in the understanding and treatment of chronic myeloid leukemia has occurred. Since CML is a disease of later years, studies analyzing the younger age groups are far and few. In addition the transcript analysis is done infrequently, mostly in adults and less commonly in children. Type of transcript has been associated with the response rates of CML patients on treatment. In children authors have reported inferior response rates in patients with e13a2 transcript as compared to e14a2 transcript. **Methods:** Investigations were performed in the Department of Hematology on the patients registered in the Department of Pediatrics and Internal Medicine. Consecutive patients 19 years or less, who were diagnosed as CML and followed up for more than one year, were studied from 2006-2012. CBC, NAP score, bone marrow examination with trephine biopsy, cytogenetic and molecular studies were done. For molecular studies RNA was extracted from 1-2 ml bone marrow aspirate in EDTA or 3-5 ml peripheral blood, followed by RT-PCR to analyze the transcripts. **Results:** We analyzed 28 patients up to the age of 19 years (pediatric and adolescent), diagnosed as CML at our institute. Their hematological profiles along with the bone marrow findings were analyzed. The phase of disease (CP, AP, BC) was classified according to the WHO 2008 criteria. The CML diagnosis was confirmed. The response to TKI was defined as per NCCN 2013 guidelines. Complete hematological response (CHR) was assessed at 3 months, and the cytogenetic response (CgR) was assessed at 12 months. One patient was in CML-BC, had e13a2 transcript and achieved CHR and MCGR. Twenty seven patients were in CML-CP, their transcript analysis with the CHR and CgR are shown in Table 1. The patients with e13a2 transcript achieved lesser CHR than the patients with e14a2 transcripts (90% vs. 94.1%). However, the patients with e13a2 transcript achieved MCGR more frequently than the e14a2 transcript patients (100% vs. 87.5%). **Table 1:** Transcript analysis vs. CHR and CgR in CML-CP patients

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Conclusions: Overall e14a2 BCR-ABL1 transcripts were detected more frequently than e13a2 transcripts. More studies with higher number of patients should be done to understand this infrequent disease in this small subset of patients.

431 ETHNIC-BASED MOLECULAR SCREENING FOR ALPHA THALASSEMA DELETIONS
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Introduction: British Columbia is one of the most ethnically diverse provinces in Canada with approximately 25% of the population immigrated from another country. In 2010, the Ministry of Health approved the reimbursement of molecular testing for alpha thalassemia. Due to limitations of resources, our laboratory was unable to establish a home-developed molecular PCR method, but resorted to purchase commercial molecular test kit for the detection of common deletion and non-deletional alpha thalassemia mutations. We also introduced ethnic-based screening by implementing voluntary questionnaires to obtain ethnic origin information that was necessary for the testing of populations at risk. Data of our test results and potential cost savings derived from our screening strategy will be presented.

Methods: All patients with physician requests for thalassemia/hemoglobinopathy investigations were required to complete ethnic origin questionnaires prior to phlebotomy. Blood samples were collected in EDTA tubes and analyzed by Sysmex XE-2100 followed by screening for beta thalassemia trait and hemoglobinopathy by capillary zone electrophoresis (Sebia Capillars). Samples with patient age <45, MCV/MCH below age-specific reference range (e.g. 80 fl/27 pg in adults), normal ferritin/iron studies and high risk ethnic origin (e.g. Southeast Asian, Mediterranean, Filipino, Thailand) were further tested by Alpha Globin Strip Assay (ViennaLab Diagnostics) after DNA extraction (Spin Micro DNA Extraction Kit, ViennaLab Diagnostics) and PCR amplification (Applied Biosystems). Results: Retrospective analysis of 34-month data between 2010 and 2013 identified 2044 alpha thalassemia molecular tests. Out of these, 1041 (51%) have an abnormal PCR result. Deletional mutations: 357 --SEA (South-East Asian), 83 --FIL (Filipino), 3 --MED (Mediterranean), 1 --THAI (Thai), 1 -alpha(20.5), 370 heterozygous for -alpha(3.7), 85 homozygous for -alpha(3.7), 28 heterozygous for -alpha(4.2). Non-deletional mutations: 14 heterozygous for Hb Constant Spring, 9 heterozygous for Hb Quong Sze, 18 anti-alpha(3.7) triplication, other uncommon mutations and compound heterozygotes. A separate analysis focusing on the ethnic-based screening strategy revealed that an estimated 15% of thalassemia investigations had not been reflexed to PCR analysis as the ethnic origin fell outside the high risk group. Applying the 15% to an estimated volume of 12,000 thalassemia investigations in the study period, approximately 1800 molecular PCR tests were eliminated that may result in potential future cost savings.

Conclusions: By using a commercial molecular test kit, our laboratory was able to identify common and uncommon alpha thalassemia mutations in a culturally diverse population. However, molecular testing for alpha thalassemia can be expensive and may not be necessary in certain patient populations. By implementing an ethnic-based screening strategy, we were able to screen for alpha thalassemia by PCR analysis on selected high risk population group and simultaneously reduced the number of unnecessary molecular tests, thus improving the overall cost-effectiveness without compromising patient care.

501 EXPERIENCE OF CONTINUING PROFESSIONAL DEVELOPMENT FOR BLOOD CELL MORPHOLOGY AND MALARIA IDENTIFICATION
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1Manchester Metropolitan University Manchester, United Kingdom, 2Manchester Royal Infirmary Manchester, United Kingdom, 3UK NEQAS (H) Watford, United Kingdom, 4Manchester University Manchester, United Kingdom

Introduction: The past decade has witnessed significant developments in digital morphology and its use for continuing professional development (CPD) for healthcare scientists. We present new developments based on the generation of image galleries, depicting multiple examples of variations in morphology, along with malaria images from different species. Four different products have been developed: peripheral blood and malaria gallery, malaria quizzes, separate peripheral blood quizzes, and the more advanced malaria education training competence (ETC).

Methods: Images for the different galleries were generated using the Zeiss Axio Imager M1 with the HRc camera. All four products, which are internet based, have been released as separate pilot projects to students and laboratory based personnel. The quizzes have so far been released in North West England. Results: The peripheral blood and malaria gallery covers basic morphology, examples including sickle cells, nucleated red blood cells, target cells and Howell Jolly bodies. Included are extensive images for each species and stage of malaria. Each gallery is composed of an icon image providing an ideal representation and a basic description, alongside 16 different examples showing variation. Each image in the gallery can be expanded to a single microscope field. The malaria and peripheral blood quizzes provide CPD certification and are available as separate packages. Participants are asked to classify cells and identify abnormalities on individual cells or microscope field images. Malaria ETC was initially developed as a Wikipedia style platform, but is now Flash based for increased security. It contains comprehensive information with images and videos covering all species and stages of malaria infection. This resource provides an in-depth training and reference package for laboratory based professionals. 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. Access will be provided for delegates to log on and assess the different products. Conclusions: All four products are now available for access, with pilot studies on-going. The peripheral blood and malaria gallery is free of charge and can be accessed by emailing pb-tag@mmu.ac.uk. The quizzes are available at a nominal charge, with wider release expected in 2014. Feedback from participants has been positive and is being used to refine the resources. Malaria ETC is available at www.haematologyetc.org.
METHODOLOGY FOR LEUKEMIA IDENTIFICATION FROM DIGITAL PERIPHERAL BLOOD CELL IMAGES
Laura Bijnor1, Santiago Alferez2, Anna Merino1, Magda Ruiz2, Luis E Mujica1, Jose Rodellar2
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Introduction: The diagnostic approach by examination of peripheral blood (PB) smear in leukemic patients provides important information that guides the diagnosis. Automatic morphological analysis systems have been developed to perform a pre-classification of the PB cells. However, while they recognize most of the blast cells, automated systems may underestimate its number mistaking them with reactive lymphoid cells (RLC). Likewise, these systems are not able to distinguish between lymphoid and myeloid blast cells. The objective of this work was to develop an automated procedure to classify the following three groups of cells: RLC, Myeloid Blast Cells and Lymphoid Blast Cells. Methods: A total of 746 digital images of individual cells from PB smears stained with May Grünwald-Giemsa and obtained in the Cellavision DM96 were analyzed. Among digital images, 309 were blasts from 13 acute myeloid leukemia patients, 263 images were from 6 acute lymphoid leukemia patients and 174 images were RLC from patients with infectious mononucleosis. A color clustering and Watershed Transformation (WT) was applied on the images in order to segment three different regions of the cell: nucleus, cytoplasm and peripheral zone around the cell. After that, mathematical morphology operations were done to improve the quality of these regions. A total of 2379 features were obtained: 2366 texture-color, 12 geometrical and 1 from the external profile of the cytoplasm. We used information theory feature selection to select the best 70 features and support vector machines (SVM) to classify the three groups. Results: The classification results using SVM with radial basis function kernel showed an overall accuracy of 86.6 %. The bold percentages in the table represent the true positives in the classification for each cell group, which were 96 % for RLC, 84.4 % for lymphoid blast cells (LBC) and 83.2 % for myeloid blast cells (MBC). Conclusions: The methodology described in this work was able to discriminate blast cells of different lineage (myeloid or lymphoid) obtaining a high accuracy classification results. As we expected, the reactive lymphoid cells were clearly separated from the blast cell groups. Further work is in progress in order to apply this methodology as a support tool for acute leukemia diagnosis.

<table>
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<th>KNOWN</th>
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<th>MBC</th>
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<td>96.0 %</td>
<td></td>
<td>4.0 %</td>
</tr>
<tr>
<td>LBC</td>
<td>1.5 %</td>
<td>84.4 %</td>
<td>14.1 %</td>
</tr>
<tr>
<td>MBC</td>
<td>3.6 %</td>
<td>13.2 %</td>
<td>83.2 %</td>
</tr>
</tbody>
</table>

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THE USEFULNESS OF SUSPECT FLAGS ON THE SYSMEX XE-2100D HEMATOLOGY ANALYZER
Samuel Comar1,2, Mariester Malvezzi1, Ricardo Pasquin1
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Introduction: Hematology analyzers generate suspect flags in the presence of morphological abnormalities and abnormal cell types. Assessing and documenting the performance of suspect flags is extremely important to the hematology laboratory, since it helps the laboratory staff on blood smears reviews. The aim or this work was to evaluate the performance of the suspect flags provided by the Sysmex XE-2100D hematology analyzer in the laboratorial routine of a large university hospital.

Methods: A total of 1739 routine samples were analyzed, including around 30% of inpatients and 70% of outpatients. The following suspect flags were evaluated: Abnormal Lymphocytes/Blasts?, Atypical Lymphocytes?; Blasts?; Immature Granulocytes?; Left Shift? and NRBC?. The evaluation was performed by comparing the occurrence of suspect flags with microscopic counterpart, for which positive smear findings were elaborated according to main guidelines and papers found in literature. The performance of the suspect flags was verified by determining the false positives (FP), false negatives (FN), true positive (TP) and true negatives (TN) rates, sensitivity (SE), specificity (SP), positive predictive value (PPV), negative predictive value (NPV), efficiency (EF), positive likelihood ratio (PLR), and negative likelihood ratio (NLR).

Results: The SE, SP and EF for the Abnormal Lymphocytes/Blasts? suspect flag was 35.59%, 95.54% and 93.50%, Atypical Lymphocytes? was 42.31%, 95.38% and 93.79%; Blasts? was 75.00%, 98.26% and 98.10%; Immature Granulocytes? was 79.17%, 95.62% and 94.71%; Left Shift? was 44.00%, 92.08% and 82.40% and NRBC? was 72.09%, 95.93% and 95.34% respectively. The PPV, NPV, PLR and NLR for the Abnormal Lymphocytes/Blasts? suspect flag was 21.88%, 97.69%, 7.97 and 0.67; Atypical Lymphocytes? was 22.00%, 98.17%, 9.16 and 0.60; Blasts? flag was 23.08%, 99.82%, 43.18 and 0.25; Immature Granulocytes? was 51.35%, 98.74%, 18.07 and 0.22; Left Shift? was 58.33%, 96.71%, 5.56 and 0.61; and NRBC? was 31.00%, 99.27%, 17.72 and 0.29 respectively.

Conclusions: In general, the performance of the main suspect flags of the Sysmex XE-2100D hematology analyzer was satisfactory and suggests that the use of the XE-2100D in routine hematology laboratory may reduce the number of blood smear reviews needed and thus increases workflow efficiency and improves turn around time by speeding up sample processing. This also means that laboratories using the XE-2100D are assisted to efficiency improvements in signaling samples with morphological abnormalities and abnormal cell types.
Introduction: Manual blood film reviews are performed after detecting abnormal counts, instrument flags or when complete blood count (CBC) results fall outside of defined criteria. Published consensus guidelines recommend slide review for a first time absolute lymphocytosis (adults >5.0 x 10^9/L, >7.0 x 10^9/L in children < 12 years old), and Atypical/Variant Lymphocyte or Blast flagging. The ability to differentiate between reactive (non-neoplastic) lymphocytes and neoplastic lymphocytes, can aid in a rapid diagnosis, crucial for initiating prompt therapeutic intervention. The Quality Management Program – Laboratory Services (QMP-LS) provides proficiency testing for peripheral blood morphology. A recent survey found laboratories experienced difficulties distinguishing reactive lymphocytes from neoplastic lymphocytes. Methods: QMP – LS distributed a peripheral blood smear obtained from a patient diagnosed with infectious mononucleosis to assess laboratory performance on white blood cell (WBC) differential count. A total of 18 laboratories correctly included reactive lymphocytes in their WBC differential count. The total WBC in this case was 10.9 x 10^9/L (Table 1), and the mean reactive lymphocyte count reported by the survey participants was 2.6 x10^9/L (27%). An additional 4 (2.3%) laboratories commented that reactive lymphocytes were present, but did not include them as a separate category in the differential count. A total of 18 (10.4%) laboratories failed to correctly report the presence of reactive lymphocytes; 3 (1.7%) reported blasts, 5 (2.9%) reported neoplastic lymphocytes, 9 (5.2%) reported both reactive lymphocytes and neoplastic lymphocytes, and 1 (0.6%) reported neither reactive lymphocytes nor neoplastic lymphocytes. 

Table 1: Laboratory Data

<table>
<thead>
<tr>
<th>Results</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukocyte count</td>
<td>10.9 x 10^9/L</td>
</tr>
<tr>
<td>Erythrocyte count</td>
<td>4.90 x 10^12/L</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>143 g/L</td>
</tr>
<tr>
<td>Hematocrit</td>
<td>0.42 L/L</td>
</tr>
<tr>
<td>Platelet count</td>
<td>40 x 10^12/L</td>
</tr>
</tbody>
</table>

Conclusions: This proficiency testing survey highlight challenges for the morphologic identification of reactive lymphocytes (Figure 1) versus neoplastic lymphocytes and reveals variation in laboratories’ reporting practice. Further education in lymphocyte morphology and standardization of reactive lymphocyte reporting practice may be useful.
respectively 10.5, 8.9 and 15.0 %. The intra-reader variation equals 25.8%. As the mean number of schistocytes per sample was 4 (range: 1–20; n=30), the mean SD was 1 schistocyte (not clinically relevant). Intra-reader variation using the manual microscope was 38.9%. Conclusions: Our study has shown that the inter-and intra-individual variation of the microscopic schistocyte count is decreased using automated microscopy. Counting schistocytes on the automated microscope is a good alternative for the traditional microscope and leads, when the observers are properly trained, to significant decrease in inter-and intra-reader variation in schistocyte count.

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EXTERNAL QUALITY ASSESSMENT SCHEME (EQAS) FOR BLOOD SMEAR INTERPRETATION: EVALUATION OF THE RESULTS AFTER THREE YEARS EXPERIENCE

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Introduction: Microscopic examination of peripheral blood (PB) cells is an important diagnostic tool. For this reason, the Spanish Haematology and Haemotherapy Society (SEHH) decided to set up a PB smear scheme. We showed the results obtained after three years experience (2011-2013). Methods: The mean number of participants was 116. A total of 18 PB films were stained with MGG and they were sent every two months: 1 Acute Promyelocytic Leukaemia (APL), 3 Hairy Cell leukaemia (HCL), 1 Sickle Cell Anaemia (SC), 2 Mantle Cell Lymphoma (MCL), 1 Sézary syndrome (SS), 5 AML, 1 ALL-B, 1 Primary Myelofibrosis (PMF), 1 Chronic Myeloid Leukemia (CML), 1 Bernard-Soulier syndrome (BS) and 1 Plasmodium falciparum (P). Patient details (age, sex, clinical data, hemoglobin, WBC, RBC and platelet counts) were included. The participants were asked to select: Up to four significant morphology features using a coding list, the diagnosis suggestion and the immunophenotypic/cytogenetic abnormalities associated to the morphology. They received a report with: 1) Morphological alterations identified by the individual participant and the referent ones 2) Frequency of the morphological alterations and diagnoses reported, 3) Clinical background of the patient including relevant immunophenotypic and cytogenetic data, and the diagnosis, 4) Photographs of the morphological abnormalities and 5) Discussion of the case. Results: The correct diagnosis for the APL survey was reported by a mean of 87 % of the participants. For the HCL surveys, hairy cells were reported by a mean of 81 %, 83 % and 94 % of the participants. A total of 94 % participants informed drepanocytes in the SC survey. The percentages in the diagnostic suggestion were not so good for the MCL and SS surveys: 51 %, 34 % and 17 %, confirming that atypical lymphoid cells are the more difficult cells to classify by conventional morphology only. Acute leukaemia surveys had very good morphological results since a mean of 99/97/96/93/90 % (AML) and 97 % (ALL) of the participants informed blast cells. A mean of 68 % and 87 % 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 the haematological disorders diagnosis and it is a useful aid for the indication of further necessary tests.

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ROUTINE BLOOD SMEAR SCAN IN LOW PLATELET COUNTS- WHAT DO WE GAIN?
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Introduction: A ‘Blood smear scan’ is one which is done to counter check the automated platelet count, when the result is flagged by the analyzer or if it is below the critical value defined by the laboratory. Professional accreditation agencies mandate such peripheral smear screening by a Hemato morphologist/Trained technician for all low platelet counts. But, this exercise is usually considered tedious; when a smear study report is not asked for, the screening is done superficially, to vouch or counter check the analyzer values. This study was conducted to assess the role of blood smear scans in adding value to the hematology reports. Methods: The study was approved by institutional ethical and research committee. A prospective study was conducted in the hematology laboratory of a tertiary care hospital during the period of January to May 2013. Complete Blood count was carried out using Sysmex KX-21 fully automated hematology analyzer. For analyzer derived platelet counts less than 100X10^9/L with or without flagging, blood smears were prepared and stained with Leishman’s stain. Those cases with low platelet counts, but with a smear study request or with a request of smear for Malarial parasite/MicroFilaria or malarial parasite positive cases in QBC were excluded from the study. With basic details and CBC report of the patient in hand, the smears were viewed by a trained Hemato morphologist. Ten fields were viewed under 10X and then selected cases were selected for microscopic examination. For analyzer derived platelet counts less than 100X10^9/L, with or without flagging, blood smears were prepared and stained with Leishman’s stain. Those cases with low platelet counts, but with a smear study request or with a request of smear for Malarial parasite/MicroFilaria or malarial parasite positive cases in QBC were excluded from the study. With basic details and CBC report of the patient in hand, the smears were viewed by a trained Hemato morphologist. Ten fields were viewed under 10X and then selected cases were selected for microscopic examination. For analyzer derived platelet counts less than 100X10^9/L. Results: A total of 964 smears were included in the study. Forty three smears (4%) were found to have normal platelet counts. Rest of the smears showed low platelet counts. Platelet estimates in about 72% of cases were low without any other identifiable abnormalities in the smear. Twenty percent of the smears showed presence of malarial parasites (Plasmodium vivax or Falciparum); About 3% of smears showed pancytopenia with or without features of other lineage abnormalities. Conclusions: In our study we found out that a routine platelet screening procedure for low platelet counts can add value to the CBC report in saving time both from physicians’ and in patients’ perspective. Furthermore, it also implies indirectly for continuous improvement of laboratory procedures with increased focus on pre analytical procedures.
Digital imaging of leukocytes in peripheral blood

1. Isolated thrombocytopenia
2. Thrombocytopenia with spleen positive for malaria
3. Normal platelet estimate (with clumps)
4. Febrycopenia with cause unknown
5. Febrycopenia with sialic cells in the smear
6. Giant platelets, pseudothrombocytopenia
7. Febrycopenia with macrocytic anemia

<table>
<thead>
<tr>
<th>Serial No.</th>
<th>No. of Smears (Percentage)</th>
<th>Finding</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>684 (71.9%)</td>
<td>Isolated thrombocytopenia</td>
</tr>
<tr>
<td>2</td>
<td>155 (20.3%)</td>
<td>Thrombocytopenia with spleen positive for malaria</td>
</tr>
<tr>
<td>3</td>
<td>43 (4%)</td>
<td>Normal platelet estimate (with clumps)</td>
</tr>
<tr>
<td>4</td>
<td>18 (1.4%)</td>
<td>Febrycopenia with cause unknown</td>
</tr>
<tr>
<td>5</td>
<td>92 (9.2%)</td>
<td>Febrycopenia with sialic cells in the smear</td>
</tr>
<tr>
<td>6</td>
<td>4 (0.4%)</td>
<td>Giant platelets, pseudothrombocytopenia</td>
</tr>
<tr>
<td>7</td>
<td>1 (0.1%)</td>
<td>Febrycopenia with macrocytic anemia</td>
</tr>
<tr>
<td>TOTAL</td>
<td>964</td>
<td></td>
</tr>
</tbody>
</table>

**TABLE 1. Details of the blood smear scan (platelet scan)**

**517 DIGITAL IMAGING**

*Jurgen Riedl*

*Albert Schweitzer Hospital Dordrecht, Netherlands*

**Introduction:** Digital imaging of leukocytes in peripheral blood smears and subsequent correct classification has been successfully implemented in various laboratories to date. Moreover it has become part of routine hematological analysis and has thereby replaced the manual differential using direct microscopy. Further studies have shown that digital imaging can be extended to different body fluids, such as abdominal, pleural and cerebral spinal fluids (CSF). An exciting new and promising feature in digital imaging is the automated recognition and subsequent classification of red blood cell abnormalities (e.g. fragmentocytes/schistocytes in TTP, target cells in thalassemia etc.). **Methods:** A total of 15-20 blood smears and 2000-4000 individual red blood cells per blood smear were analysed by the novel RBC module (Celldivision, Lund). Pre-classification and post-classification studies were performed for schistocytes/fragmentocytes, target cells, teardropcells and subsequent correlation graphs and numbers were determined. Moreover, a cohort (n=10) of blood smears of “normal” healthy individuals was also analysed to try and determine a cut-off point/value. **Results:** For schistocytes/fragmentocytes a correlation graph of y=0.8x-1 (Passing-Bablok) and a correlation coefficient of 0.95 was determined. For teardropcells a correlation graph of y=0.8x and a correlation coefficient of 0.95 was determined. The “normal” cohort of healthy individuals displayed a mean of <1% of pre-classified schistocytes. For targetcells a correlation graph of y=1.7x+4 and a correlation coefficient of 0.95 was determined. For teardropcells a correlation graph of y=0.8x and a correlation coefficient of 0.95 was determined. **Conclusions:** Digital imaging of red blood cell morphology is coming of age. Using clear cut-off points it is now possible to automatically detect and report clinical relevant morphological abnormalities of the red blood cell lineage.

**519 FRAGMENTED RED BLOOD CELL QUANTIFICATION ON CELL-DYN SAPPHIRE (ABBOTT).**

*Anneleen Schallier, Guy Van Moer, Lutgarde Smet, Serge Damiaens, Marc De Waele, Kristin Jochmans*

*UZ Brussel Brussels, Belgium*

**Introduction:** Fragmented red blood cells (FRC; schistocytes) may be found in the peripheral blood of a variety of disorders (acute kidney failure, dysfunctional mechanic heart valves, sepsis, etc) but are mainly important for the diagnosis of thrombotic microangiopathies (TMA). The ‘golden standard’ to detect these FRC is the microscopic analysis of a peripheral blood film. However, this evaluation is labor intensive and semi-quantitative. Therefore, we tested whether the CBC + retic mode on the Cell-Dyn Sapphire (Abbott) could be used as an automated screening test to detect and quantify FRC in blood samples. **Methods:** The flow cytometry mode of the Cell-Dyn Sapphire mode was used. FRC were gated out of the red blood cell (RBC) population by analyzing the scatterplot (axial light loss vs polarized side scatter), using the FCS Express program (De Novo Software). Anti-glycophorin antibody staining and dilution series of positive samples were used to validate the presence of FRC. The reference range was determined. To assess the performance of the test, we compared microscopic and automated counts of samples where the presence of schistocytes was suspected (n=133). In a last set of experiments, patients with hemoglobinopathies and RBC with membrane defects were analyzed to evaluate possible interferences of these pathologies on the measured value of the FRC. **Results:** After validation, a cut-off reference value of <0.2% of gated RBC was established. Though a good sensitivity of 97% was obtained, specificity was only 54%. The high number of false positive samples could be explained by the presence of shape anomalies of the RBC. Furthermore, all patients with hemoglobinopathies tested positive. This high degree of interference resulted in a low positive predictive value (71%). The negative predictive value was good (92%). **Conclusions:** This study validated the use of the Cell-Dyn Sapphire analyzer for the detection of schistocytes in blood samples. High sensitivity was reached and no patients with TMA were missed. When schistocyte percentages are above the cut-off value, microscopic evaluation should be performed to confirm the result. The use of this screening method for schistocytes will reduce the number of microscopic evaluations.

**521 ANALYSIS OF HAEMATOLOGICAL PARAMETERS PROVIDED BY ADVIA 2120I ANALYZER IN CHRONIC MYELOMONOCYTIC LEUKEMIA AND REACTIVE MONOCYTOSIS**

*Diego Velasco-Rodriguez, Juan Manuel Alonso-Dominguez, Nazaret del Amo, Fernando Ataúlfo González-Fernández, Natalia Acedo, Cristina Seri, Raquel Guillén, Beatriz Alvarez, Fernando Cava, Jesús Villarrubia*

*Laboratorio Central de la Comunidad de Madrid (Unilabs, BR Salud) Madrid, Spain*

**Introduction:** In a patient with monocytosis, the clinicians should consider whether the monocytosis is clonal [most of them are Chronic myelomonocytic Leukemia (CMML)] or reactive. Peripheral blood morphology of CMML is characterized by an increase of promonocytes and dysplastic features in neutrophils and monocytes. Our aim was to evaluate whether the parameters LI (lobularity index), MPX (amount of myeloperoxidase) and MNx and MNy (size and nuclear complexity respectively of mononucleated cells in the BASO channel) provided by Advia 2120i analyzer (SiemensDiagnostics®) are able to discriminate between both entities. **Methods:** 46 patients with monocytosis (monocytes >1,000/µL) were prospectively evaluated in our laboratory. Subjects with false monocytosis due to platelet clumping or hereditary partial MPX deficiency were excluded. According to several criteria [peripheral blood morphology (examined by an expert in morphologic diagnosis), coexistent chronic disease,
biochemical parameters (ferritin, reactive C protein, erythrocyte sedimentation rate) and duration of monocytes (more or less than 6 months), patients were classified in 2 groups: CMMML and Reactive Monocytosis (RM). LI, MPX, age, ferritin and classical parameters (hemoglobin, MCV, platelets, total leucocytes, neutrophils, monocytes, lymphocytes, eosinophils, basophils) were compared in both groups. Independent sample t-test was used to compare haematological parameters between CMMML and RM, and receiver operating characteristic (ROC) curves were plotted.

Results: Patients: 24 CMMML and 22 RM. Significant differences between CMMML and RM were found in: age (76.21 vs 54.73, p<0.001), neutrophils (7.82 vs 11.08, p=0.044), monocytes (2.46 vs 1.72, p=0.006), eosinophils (0.14 vs 0.45, p=0.007), platelets (194.75 vs 372.91, p=0.001), LI (1.78 vs 1.97, p=0.001) and MNy (13.10 vs 14.39, p=0.004). No differences were found in ferritin, total leucocytes, lymphocytes, basophils, hemoglobin, MCV, MPX (-2.11 vs -0.15, p=0.406) and MNx. Area under the curve of LI was 0.121.

Conclusions: LI was significantly lower in CMMML (which may reflect deficient nuclear segmentation), but it did not prove to be efficient enough to distinguish CMMML from RM. MPX was lower in CMMML, although the difference was not significant. Clinical applicability of these parameters is still to be determined, and they need to be validated in studies with larger number of people. Subjects with RM had higher leucocyte, neutrophil and eosinophil counts, and higher ferritin levels, whereas CMMML subjects had higher monocyte count and appeared to be older. Nuclear size of mononucleated cells was lower in CMMML, probably related to immature forms of monocytes.

601 DETECTION OF HCV-RNA IN PLATELETS AND ITS IMPACT ON RESPONSE TO TREATMENT IN GENOTYPE 4 PATIENTS
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1Hematology section, Laboratory Medicine and Pathology Department, Hamad General Hospital Doha, Qatar; 2Department of Health Sciences, College of Arts and Science, Qatar University Doha, Qatar; 3Department of Gastroenterology and Hepatology, Hamad General Hospital Doha, Qatar; 4Department of Gastroenterology and Hepatology, Weill Cornell Medical College, Qatar Doha, Qatar; 5Chronic Disease Epidemiology Division, Yale School of Public Health New Haven, CT, USA

Introduction: Hepatitis C virus (HCV) is one of the most important causes of chronic hepatitis globally, and particularly in Egypt, where genotype 4 (G4) prevails. Even with the recent approval of two Protease Inhibitors, the current treatment course still involves pegylated interferon and ribavirin (PEG-IFN/RBV). Detection of HCV has been reported in extrahepatic sites such as platelets. However, the complex function of platelets' HCV-RNA and platelets count in predicting response to therapies has not been well characterized. We examined the possible interplay between response to treatment with platelets and platelets' HCV-RNA in chronically HCV-G4 patients.

Methods: Twenty chronic HCV G4 patients scheduled to receive (PEG-IFN/RBV)combination therapy. Blood samples were collected pre-treatment and at weeks 4, 12, 48 and 72 post-treatment. Platelet rich plasma was prepared by centrifuging citrated whole blood samples at 150xg for 10min which was then re-centrifuged at 150xg for 10min to obtain a platelet pellet. The pellet was washed 7 times with Tyrode's solution. Paired serum and platelet pellets were then subjected to HCV/RNA extraction using the QIAPrep Viral RNA and RNeasy Mini kit (QIAGEN, Hilden, Germany). We used Quantile regression to assess relationship between SVR and platelets count, platelets and serum viral loads at baseline, and generalized estimating equation (GEE) models to examine predictors of SVR over time. Sensitivity of baseline platelets count and platelets viral load in predicting SVR was tested by ROC curve. Results: 95% of patients were men with a median age 46 (38.5-50.5) years among which 55% achieved SVR. By week 4, platelet viral load declined sharply among responders to reach approximately zero and remained ~0 throughout the follow up. In non-responders, the decline continued until week 12, then increased throughout the remaining period (Fig. 1). Decline in viral load was steeper in serum than in platelets, Platelet count remained almost constant over time among non-responders, but responders showed a steady small decline until week 12, then started to increase to pre-treatment levels. ROC curve analysis revealed that platelet viral load is a better predictor of SVR compared to serum (75% versus 54%). Only platelet viral load predicted response, such that lower platelet viral load was associated with response (z=-4.23, p<.0001). Conclusions: Pre-treatment platelet-viral load is a more sensitive predictor of SVR. Early eradication of viremia from platelet is associated with higher rate of SVR. Serum HCV RNA analysis underestimates circulating viral load and whole blood may be more sensitive.

Log platelet viral load by week and response status

603 USEFULNESS OF AN IMMATURE PLATELET FRACTION (IPF) IN THROMBOCYTOPENIA ASSESSMENT
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1 Ibnrochd university hospital casablanca, Morocco, 2 Ibnrochd university hospital casablanca, Morocco, 3 Ibnrochd university hospital casablanca, Morocco

Introduction: Thrombocytopenia that is caused by decreased production or enhanced destruction of platelets, is a common hematological abnormality and can sometimes be life threatening, to diagnosis of thrombocytopenia is difficult and it is important that enhanced destruction of platelets is differentiated from decreased production. Excepting for the bone marrow examination, that an invasive test, no simple diagnostic test exists to diagnose thrombocytopenia causes, mean platelet volume does not provide sufficient information. Methods: A new automated method to reliably quantify reticulated platelets, expressed as the immature platelet fraction (IPF), has been developed by flow cytometry techniques and can be utilized in the full blood count profile, the number of reticulated platelets reflects the rate of thrombopoiesis, increasing when platelet production rises and decreasing when production falls, in diagnosis of immune thrombocytopenia, this
parameter was reported to be useful, by recent studies. The aim of this study was to examine the usefulness of IPF measurement for differential diagnosis of thrombocytopenic disorders in our context. We prospectively enrolled in department of hematology laboratory, Ibnrochd university hospital of Casablanca 160 samples of CBC analyzed on an automated Sysmex 5000, and belonging to three groups of patients: 30 with confirmed peripheral thrombocytopenia, 30 central thrombocytopenia and 100 samples of healthy subjects. Results: for peripheral The first results of this work showed a reference rate of IPF 2.25% (from 0.5 to 6%) in the healthy group, 17.1% (8.6 to 37.5%) in the patients followed thrombocytopenia and reaches an average 2.10% (0.5, 4%) in central thrombocytopenia, followed by two patients in this treatment group showed a progressive decrease in parallel with IPF correction of platelet counts. Conclusions: These results show that the IPF, as reported in the literature, rapid setting and inexpensive presents valuable test in the etiologic diagnosis of thrombocytopenia and must have its place as a standard test in the investigation of thrombocytopenic patients.

605 THE EFFECTS AND IMPLICATIONS OF A PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR R LIGAND ON PLATELET DIFFERENTIATION AND ACTIVATION
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1Department of Laboratory Medicine, Dong-A University College Of Medicine Busan, South Korea, 2Department of Biochemistry, Dong-A University College Of Medicine Busan, South Korea

Introduction: Recently, the ligand-activated transcription factor peroxisome proliferator-activated receptor gamma (PPARγ) was to promote the formation of platelets from megakaryocytes and accelerate platelet recovery after radiation-induced bone marrow injuries. The endogenous prostaglandin 15-deoxy-D12,14-prostaglandin J2 (15d-PGJ2) is a ligand of PPARγ. In the present study, we investigated whether the PPARγ ligand and its antagonist affected platelet function in vitro, and how. Methods: Platelets were obtained from plateletpheresis products by centrifugation. DAMI and MEG1 cells were used as well. Western blotting for PPARγ was performed, and bands were visualized by chemiluminescence. The effects of the potent PPARγ antagonist GW9662 and the anti-oxidant brown algae-based polyphenol complexes Seanol and Fucoidan were also evaluated. Platelet flow cytometry was performed after each treatment to analyze the effects of the treatment on platelets. Results: Treatment with 10 uM 15d-PGJ2 significantly affected platelet expression of CD42b (decreased) and CD62P (increased), as measured by flow cytometry. At 20 uM, GW9662 did not effect any significant changes, whereas 25 ug/mL Seanol and 50 ug/mL Fucoidan both increased CD63 and PAC1 expression. Studies on the effects of these treatments on DAMI and MEG1 cells revealed clearly different outcomes as compared with that of mature platelets. Conclusions: The mechanism of platelet production and activation is not fully understood. Understanding the mechanisms that underlie megakaryocyte maturation and platelet production will provide insight into new methods of enhancing the production of platelets from precursor cells. Our findings support the hypothesis that PPARγ plays a role in platelet activation, and we are currently planning to investigate its involvement in areas including thrombopoiesis from cord blood and diseases such as myelodysplastic syndromes.

607 THEN AND NOW: RETICULATED PLATELET ENUMERATION IN PATIENTS WITH THROMBOCYTOPENIA
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1Division of Hematology, London Health Sciences Centre London, ON, Canada, 2Abbott Diagnostics Santa Clara, CA, USA

Introduction: Diagnosing the cause of thrombocytopenia often requires a bone marrow aspiration, an invasive procedure. Reticulated platelets (rPLTs) are to platelets as reticulocytes are to red blood cells (RBCs). Automated complete-blood-count analyzers offer reliable, accurate, and precise enumeration of reticulocytes that may provide useful information in the differential diagnosis of anemia. In the differential diagnosis of thrombocytopenia accurate rPLT has yet to be achieved, partially due to the lack of a robust reference method. Previous work focused on either accurate enumeration of platelets (low end <50x10^3/L) or determination of rPLT percentage. Our goals were to re-examine previous work and evaluate dyes that may offer the same ability to distinguish rPLTs from mature platelets as reticulocytes can be from RBCs, while incorporating accurate platelet enumeration into the analysis. Methods: After reviewing previously published studies on Thiazole Orange (TO) staining of rPLTs , we systematically evaluated CD41/CD61 in combination with TO (BD Biosciences) with serial dilutions, incubation times, stopping reagents, and gating strategies. rPLT methods have not taken advantage of platelet enumeration and for this reason we decided to incorporate the ISLH platelet enumeration into this method. We also evaluated novel dyes that may provide better discrimination between immature and mature platelets. Results: TO has a very high quantum yield upon binding to RNA, however both staining of residual RNA in mature platelets and non-specific, RNA independent, staining has previously been well documented. Gating strategy to determine rPLT fraction can be done in various ways using either unstained control samples or internal standards. Optimal TO concentration was determined to be 10% of stock concentration. Incubation time studies at various TO concentrations determined optimal time to be 30min. Fixation was achieved with 1% formaldehyde. Syber-Green II and other organic dyes were investigated, however background issues similar to those of TO were seen. Platelet enumeration was consistent with our previous studies in this area. Conclusions: A combination strategy, platelet enumeration with rPLT percentage, is possible. TO is an effective rPLT dye. Although not optimal, TO stains immature platelets with low signal to noise compared to mature platelets. Accurate rPLT percentage requires an effective gating strategy, due to background fluorescence cursor placement becomes an important issue when determining the positive fraction. Enumeration of rPLT percentage combined with an accurate platelet count, particularly in the low range, corresponds to the degree of production within the bone marrow giving the physician an effective early indicator of therapy.
609 EVALUATION OF PLATELET INDICES IN PREGNANT WOMEN WITH HYPERTENSIVE DISEASE
Daniela Moraes1, Bartira E. Pinheiro da Costa1, Terezinha P. Munhoz2, Carlos E. Poli-de-Figueiredo2
1Hospital São Lucas da PUCRS Porto Alegre, Brazil, 2Faculdade de Farmácia PUCRS Porto Alegre, Brazil

Introduction: During pregnancy imbalance of the hemostatic mechanism may occur with elevated levels of coagulation factors, tending to hypercoagulability with increased risk of thrombosis. In pregnant women with hypertensive disorder, especially in preeclampsia, there is a decrease in the total platelets number as well as changes in platelet indices, mean platelet volume (MPV) and immature platelet fraction (IPF). The IPF has been suggested as a sensitive parameter to monitor changes in platelet production. An automated technique for obtaining this index has been used. The aim of this study was to analyze the behavior of the immature platelet fraction using fluorescent flow cytometry in patients with gestational hypertension. Methods: A cross-sectional study was conducted with 99 pregnant women to estimate IPF levels in maternal blood in preeclampsia syndrome (SPE), in gestational hypertension without proteinuria (GH), and normal pregnancy (NP) attended at São Lucas Hospital/PUCRS, Porto Alegre, Brazil. Following ethical approval and informed written consent, samples were taken from 34 SPE and 32 PNP and 33 NP. IPF levels were measured using the XE-5000 (Sysmex Corporation, Kobe, Japan) with fluorescent flow cytometry methodology. Results: None of parameters followed like: hemoglobin level, red blood cell, white blood cell and platelet count were significantly different between women with PES, GH and NP (P>0.05). The IPF count, MPV and PDW in pregnant women with preeclampsia syndrome, hypertensive and normotensive was statistically different between groups (P<0.001). Conclusions: Platelet activation distinction profile between SPE, GH and NP was detected. Due to the new method facility, it could be useful to the early diagnosis of preeclampsia.

611 PLATELET VOLUME INDICES AND PLATELET COUNT IN PATIENTS WITH ACUTE CORONARY SYNDROME
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1Alzaiem Alzahari University Khartoum, Sudan, 2Sharq Elniel college Khartoum Sudan

Introduction: Acute coronary syndromes (ACS) are a set of signs and symptoms due to the rupture of a plaque and are a consequence of platelet-rich coronary thrombus formation. Platelets and their activities have an important role in initiation of atherosclerotic lesions and coronary thrombus formation. The study aimed to study the changes in platelet volume indices and platelet count in acute coronary syndrome in order to assess their usefulness in predicting coronary events. Methods: This was descriptive comparative study of 120 patients with acute coronary syndrome 60 patients with un stable angina(UA), 60 with myocardial infarction (MI) and 60 matched healthy controls with no history of heart disease and a normal electrocardiogram, venous blood sample were drawn from all subjects on admission and collected in EDTA evacuated tubes. Platelet count and volume indices were assayed using Sysmex KX21-N haematology nalyzer. Results: The platelet count was significantly lower in patients with acute coronary syndrome (patients with MI 259.75×10⁹/L and UA 264.00×10⁹/L) as compared with healthy population 310.65×10⁹/L. The mean platelet volume was significantly higher in patients with MI 9.8fl , UA 9.8 fl compared with normal population 9.1 fl(p<0.001). Conclusions: Patients with acute coronary syndrome had higher platelet volume indices and lower platelet counts compared with the normal population; larger platelets are more active and are a risk factor for developing coronary thrombosis leading to myocardial infarction. So measurements of platelet volume indices and platelet count might give some benefit in detecting those patients at higher risk for acute coronary events.

613 PLATELET COUNT ON BECKMAN COULTER DXH800 CELLULAR ANALYSIS SYSTEM: COMPARISON WITH FLOW REFERENCE METHOD.
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Centre Hospitalier De Troyes, Troyes, France

Introduction: A precise and accurate platelet count is important in both clinical hematology and platelet research laboratories. The increases in platelet transfusions and thrombopoietin therapy further emphasize the importance of platelet counts. Pathological samples may challenge instrument platelet counts analyzed on routine hematology systems; these samples may be identified with “R” flag for review. Our study compared the performance of platelets flagged “R” on the UniCel DxH 800 Coulter Cellular Analysis System (Beckman Coulter) to the platelet flow reference method. The platelet values (PLT) were in the low to low-normal clinical range. Methods: 81 blood samples from hospital routine collected between June and December 2013 were included in the study. Venous whole blood was collected in a salt of EDTA and analyzed within 12 hours of collection. Only samples with PLT “R” flags on the DxH 800 were included in the study. The reference method was Flow Cytometry analysis with platelet markers CD41 and CD61, performed according to the ICSH working reference method. Statistical analysis was performed using MedCalc software (Ostend, Belgium). Results: The PLT performance characteristics obtained by both methods for samples flagged with “R” by the DxH 800 System are presented in Table 1 (n=81). The correlation coefficient R was 0.8675 (95% confidence interval 0.8009 - 0.9130). We further analyzed a subgroup of PLT less than 20x10⁹/μl where the correlation between the DxH 800 and Flow was perfect (y=x), but the number of samples was very low (n=6). Table 1

<table>
<thead>
<tr>
<th>Method</th>
<th>Mean x10⁹/μl</th>
<th>Median x10⁹/μl</th>
<th>Minimum x10⁹/μl</th>
<th>Maximum x10⁹/μl</th>
<th>Normal Distr.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flow</td>
<td>68</td>
<td>64</td>
<td>2</td>
<td>144</td>
<td>0.0037</td>
</tr>
<tr>
<td>DxH</td>
<td>52</td>
<td>55</td>
<td>2</td>
<td>98</td>
<td>0.0123</td>
</tr>
</tbody>
</table>

Conclusions: Our results indicate that for the samples flagged with “R” flag for PLT count, the DxH 800 is able to provide results consistent with the flow reference method even at a clinically important threshold (PLT < 20x10⁹/μl). We observed an underestimation of the platelet count on the entire dataset of flagged samples. PLT studies need to be performed at this low clinical range on unflagged samples.
PLATELET FUNCTION BUT NOT PLATELET COUNT CAN BE ASSESSED IN HIRUDIN WHOLE BLOOD

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Centre for Haemophilia and Thrombosis, Department of Clinical Biochemistry, Aarhus University Hospital Aarhus, Denmark

Introduction: Platelet function determined using the Multiplate® Analyzer is recommended to be performed in hirudin anticoagulated blood. The platelet function in hirudin whole blood has been demonstrated stable for up to two hours. Platelet count is usually assessed using EDTA anticoagulated blood, but the stability of platelet count in hirudin is still undisclosed. The present study aimed to investigate the stability of platelet count in hirudin whole blood in order to facilitate experimental research including platelet function. Methods: Hirudin (r-hirudin >15 µg/ml) and EDTA (K3-EDTA 7.2 mg) blood samples were obtained from 12 healthy volunteers. Analysis of platelet count was performed immediately and one, two and four hours after blood sampling. Platelet count was measured by optical and impedance analysis using a routine haematological analyzer (Sysmex XE-5000) and by flow cytometric analysis (Navios, Beckman Coulter), applying immunofluorescent antibodies according to the international reference method. Finally, visual evaluation was performed in three samples using manual platelet counting. Statistics were performed using repeated measures analysis of variance (ANOVA) and paired student’s t-test. Results: Routine optical and impedance platelet counting in hirudin was not stable, and a significant deterioration was observed already one hour after blood sampling (p-values <0.03). However, platelet counts in hirudin were stable for one hour using the flow cytometric reference method (p=0.37). Platelet count in hirudin was significantly reduced after two hours using all methods (p-values <0.003). Platelet count in EDTA was stable for four hours using the optical routine analyzer (p=0.87) and the flow cytometric reference method (p=0.32). Of note, platelet count displayed a small but significant increase over time when looking at the impedance measurements in EDTA (p<0.01). Manual platelet count suggested micro-aggregate formations in hirudin, but not in EDTA. Conclusions: The platelet count shows limited stability in hirudin blood and cannot be assessed applying a routine hematology analyzer. However, using the international reference method the platelet count can be determined in hirudin for up to one hour after blood sampling. The platelet function in hirudin whole blood has previously been shown stable for up to two hours, but assessment of the platelet count on the same sample may be misleading.

RED BLOOD CELL INDICES IN NIGERIAN MALARIA PATIENTS

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¹Madonna University Elele, Nigeria, ²College of Health Technology Ogbia Nigeria

Introduction: Malaria is transmitted by the bite of infected Anopheles mosquito. It is the most medically-important parasitic disease of humans. Methods: Six hundred and thirty (630) patients attending clinic at Madonna University Teaching Hospital (MUTH) Elele, were recruited for this study. Verbal consent was obtained prior to sample collection. The samples were analyzed for malaria parasite (MP), packed cell volume (PCV), Haemoglobin (Hb), Red blood cell count (RBC), Reticulocyte count, Mean cell volume (MCV), Mean cell haemoglobin (MCH) and Mean cell haemoglobin concentration (MCHC) using standard manual methods. Results: The result shows that 200 patients (32%) were apparently malaria parasite negative (aparastasia) while 430 patients (68%) were malaria parasite positive (parastasia). The positivity ranges from 1+ to 3+. Table 1: Distribution of positive cases

<table>
<thead>
<tr>
<th>Positivity</th>
<th>Number</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 +</td>
<td>250</td>
<td>58.1</td>
</tr>
<tr>
<td>2 +</td>
<td>150</td>
<td>34.9</td>
</tr>
<tr>
<td>3 +</td>
<td>30</td>
<td>7.0</td>
</tr>
<tr>
<td>Total</td>
<td>430</td>
<td>100</td>
</tr>
</tbody>
</table>

There was statistical significant (p<0.05) decrease in PCV, Hb, and RBC while Reticulocyte count, MCV and MCH had statistically significant (p<0.05) increase according to table 2.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Parasitic subjects</th>
<th>Non-parasitic subjects</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCV (%)</td>
<td>35.06±0.01</td>
<td>39.25±2.82</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Hb (g/dl)</td>
<td>11.51±2.04</td>
<td>13.32±1.65</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Retic count (%)</td>
<td>2.04±0.72</td>
<td>3.13±0.23</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>RBC (x10⁹/l)</td>
<td>3.86±0.96</td>
<td>4.60±0.29</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>93.52±13.55</td>
<td>85.84±3.52</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>30.61±4.39</td>
<td>29.04±1.15</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>MCHC (g/dl)</td>
<td>32.77±1.01</td>
<td>33.95±0.87</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Conclusions: These changes show that red cell production in malaria infested patients is reduced. This work therefore advocates for the use of haematinics in the treatment and management of Nigerian malaria patients.

QUALITY CONTROL OF CAPILLARY ELECTROPHORESIS CAPILLARYS² IN DIAGNOSIS OF HAEMOGLOBINOPATHIES

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¹Hemobiology Service Chu Oran Alegria Oran, Algeria

Introduction: capillary electrophoresis Capillarys² is designed for the separation of normal hemoglobins (A, F and A2) and for the detection of common hemoglobin variants (including S, C, E and D), but the quality control of the results is necessary to validate this method and give a reliable diagnosis of haemoglobinopathies. Methods: The 06 month prospective study was to assess the capillary electrophoresis fluid stream by the CAPILLARYS®/2 Sebia laboratory; 1) internal quality controls: In our study the internal quality controls was made with: CONTROL NORMAL Hb , the control sample contains two types of fractions: Hb A : 97.3 % ± 0.5 HbA 2 : 2.7 % ± 0.5 . after we obtained and analyzed the graph LEVEY JENNINGS 2 evaluation of precision (repeatability intra-assay and inter-assay reproducibility): a) repeatability intra-assay : Three different blood samples were analyzed with the system CAPILLARYS kit with the same lot of assay buffer. Sample 1: sample rate to normal Hb A2 (normal subject). Sample 2: Sample with increased Hb A2 levels (blood of a patient with beta thalassemia heterozygotes). Sample 3: Sample with abnormal hemoglobin “Hbs,” corresponding to the blood of a patient with sickle cell trait. Each sample was analyzed in 5 capillary .
b) reproducibility inter-assay: the same that repeatability but each sample was repeated 5 times.

**Results:** Interassay precision and interassay reproducibility:

CV (coefficient of variation) repeatability of fraction A Hemoglobin does not exceed 0.23%, the fraction A2 does not exceed 1.6%. The average CV of the reproducibility of the fraction A Hemoglobin is 0.07%, that of A2 fraction is 1.7%.

2) Evaluation of precision, repeatability intra-assay and inter-assay reproducibility:

Evaluation of the fidelity of CAPILLARYS showed that intra-test repeatability and inter-assay reproducibility was excellent for all hemoglobin fractions with an average of 0.69% CV (<1.7%).

**705 ALLOIMMUNIZATION IN TRANSFUSED SICKLE CELL PATIENTS: EFFECT OF RBC ANTIGEN MATCHING**

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2) University of Michigan Michigan, MI, USA,
3) University of Leiden Leiden, Netherlands

**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 antigen 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 matched were phenotypically matched for ABO, RhD and C,E and K antigens if patient had no previous antibodies. If patients had known antibody(ies) the units were matched for C,E,K,Fyα,Fyβ,Jkα and Jkβ. Results: A total of 105 SCD patients (79 SS, 11 SC, 8Sβthal 4 and 7Sβthal 1), 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:** Phnetotyping matching of RBC in sickle cell patients reduced alloiminnization by about 50 percent.

**707 MCV FLAG- AN INDEPENDENT SCREENING MARKER FOR HEMOGLOBINOPATHY**

Srinivas Chakravarthy, Vijayakumar Valappil K, Mathivanan Durairaj, Mohandas PVA

MIOT Hospitals Chennai, India

**Introduction:** Hemoglobinopathy is a varied disorder and its presentation differs. The hemoglobinopathy screening is important procedure during pre-operative procedures, Antenatal cases and premartial cases. Currently we have many formulas and smear study which will help us pick up these few Abnormalities. But all are not always successful in picking the hemoglobin abnormality. In our centre we wanted to explore whether MCV alone can be a screening marker for the hemoglobinopathy.

**Methods:** We collected data from 200 cases for a period of one year (Jan-Dec 2013) who had undergone Capillary electrophoresis, HPLC and CBC. The CBC were analysed using Sysmex 4000i along with the smear study. The Blood was next subjected to HPLC using Biorad Variant D10 machine. The samples were later put on Sebia Capillary electrophoresis machine. All the CBC sample with Normal hemoglobin with MCV flag(-) along with other unaltered red cell indices were screened for abnormalities of red cell by smear study. Rest of the samples with low hemoglobin and low red cell indice with erythrocytosis were also scrutinised for red cell abnormality. The Abnormalities noted on microscopy were noted along with HPLC finding and Capillary electrophoresis pattern.

**Results:** 108/200 cases were found to have normal hemoglobin pattern. The CBC along with Smear study and Iron profile were
correlated. In 92 cases we found that there was detection of abnormal hemoglobin with a Hb range of 9.5-10.7 g/dl. The mean being 12gms%. None of the RBC indices were abnormal except for the flagged MCV(MCV-). The CBC was run on sysmex 4000. The Mark of “-” next to MCV which was normal was peculiar and these cases were found to have abnormal hemoglobin. The Mark corresponded to MCV values ranging between 79.7- 81.8fl. Out of 68 cases, 50 cases were having Beta Thalassemia Triat, 5 cases had HbC, 5 Cases had Mild elevated HbF and 3 cases had hemoglobin D. Conclusions: 1. Normal Red cell parameters with flag MCV(-) is an indicator of Hemogloblinopathy 2. Normal Hemoglobin with near normal MCV along with normal MCH, MCHC and Normal smear study patients should be screened for Hemogloblinopathy using at least HPLC. If HPLC reveals abnormality it should be confirmed by Capillary electrophoresis. 3. The HPLC and Capillary electrophoresis are two complementary technology to confirm certain hemogloblinopathy and hence it is recommended to have in any labs performing screening for hemoglobinopathy.

709 HAEMOGLOBIN AND HAEMATOCRIT: IS THE THREEFOLD CONVERSION VALID?
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Introduction: It has been reported that the standard threefold conversion from haematocrit to haemoglobin understimates the prevalence of anaemia in diverse settings. Methods: The effectiveness of this conversion was determined by comparing the values of haemoglobin concentration obtained with modified azidemethaemoglobin (Hemocue) method and those obtained by dividing the haematocrit by 3. Samples and data from 100 apparently healthy adults, aged between 18 - 50 years and consisting of 77 males and 23 females were included in this analysis. Results: Significant difference was observed (p=0.012) for values of haemoglobin concentration derived from haematocrit (11.6 ± 2.45 g/dl) against those obtained from modified azidemethaemoglobin method (10.90 ± 2.43 g/dl). Conclusions: It was concluded that the use of haemoglobin derived from haematocrit could underestimate the prevalence of anaemia in our setting. Therefore, direct determination of haemoglobin should be the measurement of choice.

711 CONFIRMATION OF HBF ON AUTOMATED HEMOGLOBIN ANALYZER USING RAPID HB F TEST
Goonnapa Fucharoen, Wachiraporn Tawean, Supan Fucharoen
Centre For Research And Development Of Medical Diagnostic Laboratories KHON KAEN, Thailand

Introduction: Many Hb variants are co-separated with Hb F on automated Hb analyzer leading to misdiagnosis at such as Hbs Tak, Q-Thailand by capillary electrophoresis, Hbs Hope, Cook and Phimai by HPLC. We have developed a rapid Hb F test for confirmation of this in routine setting. Methods: This test is based on a modified alkaline denaturation. Briefly, 20 microliter of whole blood was made hemolysis by 500 microliter of distilled water. Twenty microliter of alkali solution was added and denatured Hb was made precipitated with 500 microliter of salt solution. After centrifugation, Hb F which is resistant to alkaline appears as brown color in the supernatant fluid and could be inspected by necked eye. No color appearance in the supernatant fluid indicates non Hb F. Results: All samples of beta-thalassemia/ Hb E (N = 20), homozygous beta-thalassemia (N = 1) delta beta-thalassemia (N = 4) and hereditary persistent of fetal Hb (HPFH) (N = 6) commonly encountered in our laboratory were positive by this rapid Hb F test, the amounts of Hb F varied from 15.4-97.7 %. In contrast, samples with Hb variants resembling Hb F such as Tak (N = 4), Q-Thailand (N = 2), Hope (N = 10) were negative. Conclusions: This rapid Hb F test is simple and proved useful for confirmation of Hb F detected by automated Hb analyzer in routine setting.

713 PROVIDING APPROPRIATE GENETIC INFORMATION TO HEALTHY MULTI-ETHNIC CARRIERS OF HEMOGLOBINOPATHY IN THE NETHERLANDS
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1Hemoglobinopathies Laboratory, dept. of Clinical Genetics, Leiden University Medical Center Leiden, Netherlands, 2Associazione Nazionale Microcitemie Italia (ANMI ONLUS), Centro Studi Microcitemie di Roma (CSMR). Rome, Italy

Introduction: The aim of this study was to enquire whether informing healthy hemoglobinopathy carriers about their condition, is a welcome initiative in The Netherlands. In addition, it was studied whether using information letters and thorough explanation is associated with presence or absence of undesired feelings or emotions. Methods: We have approached 100 multi-ethnic carriers previously diagnosed in our lab. All subjects had previously received our information letter through their physician who was supposed to have provided an explanation of the letter if required. We have enquired whether the subjects had experienced negative or positive emotions after receiving our diagnosis and explanation and to which degree, if they were sufficiently informed and satisfied and if they would have considered prevention in case of risk. The rate negative versus positive feelings was calculated using a numerical distribution. Results: We have registered negative feelings in a rate that was directly proportional to the lack of information. While the number of registered negative feelings in well informed carriers was very low it was more present in badly informed. Nevertheless, all participants found carrier information a welcome initiative and over 80% of them declared to be in favor of prenatal diagnosis in case of risk. Conclusions: Carrier information is essential for an informed reproductive choice and is welcome in a multi-ethnic society. Unfortunately, information is not always consequently provided and should therefore be imbedded in the ongoing national screening for Rhesus and infectiousdiseases available to all women in early pregnancy.

715 HEMOGLOBINOPATHIES DIAGNOSTICS IN THE NETHERLANDS: EXPERIENCES OF THE REFERENCE LABORATORY
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Hemoglobinopathies Laboratory, dept. of Clinical Genetics, Leiden University Medical Center Leiden, Netherlands

Introduction: As a consequence of immigration, occurrence of the hemoglobinopathies has increased during the last three decades in The Netherlands (approx. 1:3000 newborns). Besides mutations introduced in this way, mutations endemic to the autochthonous
Dutch population are present. Obviously, the incidence of these mutations is extremely low due to the absence of *malaria tropica* as selective pressure. The description of the clinical effects of these exotic hemoglobin variants and thalassemias is important because lack of knowledge may hamper prediction of risk in genetic counseling of mixed couples. Multi-ethnic societies require particular attention for the genetic burden of hemoglobinopathies (HbP). In a mixed couple of whom one of the partners is autochthonous, it is not sufficient to consider carrier analysis obsolete based on ethnic background. This poster gives an overview of Hb variants and thalassemia mutations found in Dutch Caucasians during the last few decades during routine HbP-screening.

### 717
**OSMOTIC GRADIENT EKTACYTOMETRY BY LASER-ASSISTED OPTICAL ROTATIONAL CELL ANALYZER (LoRRca) IN THE DIAGNOSIS OF RED BLOOD CELL MEMBRANE DISORDERS.**
Albert Huisman¹, Brigitte van Oirschot², Wouter van Solinge³, Richard van Wijk⁴
¹University Medical Center Utrecht, Utrecht, Netherlands

**Introduction:** Hereditary disorders of the red blood cell (RBC) membrane constitute a major cause of hereditary hemolytic anemia. It concerns a heterogeneous group of diseases with highly variable clinical expression, which can be challenging to diagnose. Traditionally RBC membrane disorders are classified according to the morphological appearance on a blood smear. Hereditary spherocytosis (HS) and elliptocytosis (HE) are among the more common abnormalities, whereas hereditary pyropoikilocytosis (HPP) and stomatocytosis (HST) are more rare. HS, HE, and HPP is caused by mutations disrupting the RBC cytoskeleton or its anchoring to the RBC membrane. HST results from disturbed ion homeostasis. Inheritance of these diseases is in most cases autosomal dominant. **Methods:** A number of diagnostics tests are available for the diagnosis of RBC membrane disorders. The golden standard however is osmotic gradient ektacytometry. Basically, this technique measures red blood cell deformability under stress and during gradually changing osmotic conditions. Deformability is expressed as the elongation index (EI). The introduction of the Laser-assisted Optical Rotational Cell Analyzer (LoRRca, Mechatronis, Hoorn) has recently re-introduced this technique to the clinical laboratory. We have implemented the LoRRca in our laboratory for the diagnosis of RBC membrane disorders. **Results:** Normal osmotic gradient ektacytometry curves were obtained from 40 healthy control subjects. Table 1 lists the reference ranges for our laboratory for the three informative parameters: $O_{\text{max}}$ (osmotic value at which the EI reaches its maximum, reflecting cellular surface area-to-volume ratio), $E_{\text{max}}$ (maximal deformability, reflecting mean surface area), and $O_{\text{hyper}}$ (osmotic value at which the EI reaches half of its maximum value, reflecting cellular hydration status). When applied to patient samples and compared to a daily fresh control sample a number of different RBC membrane disorders can be distinguished. Figure 1 shows some typical patient examples (red curve), including HS (decreased $E_{\text{max}}$ and $O_{\text{hyper}}$, increased $O_{\text{max}}$), HPP (decreased $E_{\text{max}}$ and trapezoidal-shaped curve), and hereditary xerocytosis or dehydrated HST (left-shift of the curve). **Table 1.**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>$O_{\text{max}}$</th>
<th>$E_{\text{max}}$</th>
<th>$O_{\text{hyper}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference range</td>
<td>151-175</td>
<td>0.586-0.610</td>
<td>473-534</td>
</tr>
</tbody>
</table>

**Conclusions:** Osmotic gradient ektacytometry by Laser-assisted Optical Rotational Cell Analyzer (LoRRca) represents a feasible and reliable diagnostic method to diagnose various RBC membrane disorders. We are currently exploring the added value of other parameters such as AUC (area under the curve), in particular with regard to phenotypic severity. Furthermore, we are currently investigating the applicability of osmotic gradient ektacytometry in other RBC disorders such as sickle cell anemia, thalassemia, and enzymopathies.

### 719
**HEMATOLOGIC FEATURES OF THALASSEMIC LAOTIAN-NEWBORNS: APPLICABILITY OF THE MEAN CORPUSCULAR VOLUME AND MEAN CORPUSCULAR HEMOGLOBIN FOR ALPHA-(0)-THALASSEMIA SCREENING IN A SETTING WITH LIMITED RESOURCES**
Chanhom Khonsavanh¹, Pattara Sanchaisuriya², Kasama Wongprachum¹, Virak Vidamaly³, Boulay Norcharoen⁴, Kanokwan Sanchaisuriya⁵, Frank Schelp⁶
¹Graduate School, Khon Kaen University Khon Kaen, Thailand,
²Maria Teresa Hospital Vientiane, Laos, ³Department of Nutrition, Faculty of Public Health, Khon Kaen University Khon Kaen, Thailand, ⁴Centre for Research and Development of Medical Diagnostic Laboratories (CMDL), Faculty of Associated Medical Sciences, Khon Kaen University Khon Kaen, Thailand

**Introduction:** Alpha-(0)-thalassemia, a severe form of thalassemia, is highly prevalent in Lao People Democratic Republic (Lao PDR). Neonatal screening is one of the options to control the disease. In this study, hematologic features of Laotian newborns with different forms of thalassemia were determined. The mean corpuscular volume (MCV) and the mean corpuscular hemoglobin (MCH) were validated for screening of alpha-(0)-thalassemia. **Methods:** A cross-sectional study was conducted at Maria Teresa Hospital, Lao PDR. A total of 265 cord blood samples from unrelated Laotian newborns were collected. Hematologic features were determined using an automated blood cell analyzer. Hemoglobin (Hb) profiles were investigated using either cellulose acetate electrophoresis or an automated Hb analyzer. Identification of Hb E gene and common alpha-thalassemia mutations including alpha-(0)-thalassemia (SEA & THAI deletion), alpha-(+)-thalassemia (3.7 & 4.2 kb deletion), Hb Constant Spring (Hb CS) and Hb Pakse' was done using PCR-based techniques. **Results:** The incidence of alpha-(0)-thalassemia among 265 Laotian newborns was 14.3%. Other forms of thalassemia included 26.3% Hb E, 10.6% alpha-(+)-thalassemia, 6.4% Hb CS, and 0.4% Hb Pakse’. Hematologic analysis revealed...
a reduction in MCV and MCH for all forms of alpha-thalassemia including among alpha-thalassemia individuals co-inherited with Hb E. Using MCV < 95 fl or MCH < 30 pg as cutoff value, the sensitivity of 97.3% and specificity of 76.7-86.8% with the positive predictive values (PPV) of 41.1-55.2% were achieved. However, when MCV < 95 fl and MCH < 30 pg were used in combination, the specificity increased to 92.1% and the PPV to 67.3%. Conclusions: In a setting with limited resources, the application of MCV < 95 fl in combination with MCH < 30 pg might be used as a primary screening tool for alpha-(0)-thalassemia in newborns.

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HAEMOLYTIC DISEASE OF THE FETUS AND NEWBORN (HDFN) AMONG SUDANESE JAUNDICED NEWBORN ATTENDING OMDURMAN MATERNITY HOSPITAL IN SUDAN
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Introduction: Hemolytic Disease of the Newborn (HDN), also known as erythroblastosis fetalis, is immunization, or blood group incompatibility, occurs when fetal red blood cells (RBCs), which possess an antigen that the mother lacks, cross the placenta into the maternal circulation, where they stimulate antibody production. The antibodies return to the fetal circulation and result in RBC destruction. The aim of the present study was to provide data on the frequency of hemolytic disease of the fetus and newborn among Sudanese jaundiced newborn attending Omdurman maternity hospital. Methods: This was prospective cross sectional hospital based study was conducted between April and June 2013, on 220 individuals (110 mothers and their 110 jaundiced newborn) admitted to Omdurman maternity hospital. We performed the direct coomb’s test (DAT) for the jaundiced newborn and indirect antiglobulin test (IAT) for their mothers in addition to identifying their blood group. For any ABO incompatibility we screen the baby serum or plasma for the presence of hyper immune IgG anti A and anti B. Hb , PCV. Reticulocyte count and blood films were also had been done to confirm the hemolytic state. Results: The frequency of hemolytic disease of the fetus and newborn was found to be (57.3%), of which HDFN due to non D antigen (29.1%), HDFN due to ABO incompatibility (16.4%) and HDFN due to Rh D incompatibility (11.8%). Conclusions: The study concluded that, despite prophylactic use of Rh immunoglobulins, anti-D is still one of the common causes of HDFN, Moreover, antenatal assessment need to be done routinely in Sudan in order to predict earlier the occurrence of HDFN inorder to reduce the clinical significance of the disease.

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PEDIATRIC REFERENCE RANGE STUDY FOR CELL-DYN SAPPHIRE MCVr, MCHR, MCHCr
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Introduction: Pediatric iron deficiency anemia (IDA) is the most common nutritional deficiency in the world, as per the World Health Organization (WHO), affecting 47% of preschool-age and 25% of school-age children. IDA remains a public health issue in the US especially among high risk populations including children living in poverty. Studies have indicated iron deficiency may be the primary cause of irreversible developmental and psychomotor deficiencies, mild to moderate decreases in immune function, and increases in intestinal absorption of lead. The laboratory assessment of IDA has traditionally consisted of performing the complete blood count (CBC) to establish the levels of Hgb, Hct and MCV. The American Academy of Pediatrics has defined anemia when the Hgb level is less than 11g/dL (110 g/L). Routine availability of extended reticulocyte parameters from modern hematology analyzers has provided new information in monitoring erythropoiesis. The MCVr, MCHR and MCHCr are cell by cell measurements of reticulocyte size and hemoglobin content and have been reported as indicators of early onset anemia. The goal of this study is to determine pediatric reference intervals for these new parameters as reported on the Abbott CELL-DYN Sapphire hematology analyzer. Methods: Testing of residual bloods from ordered CBCs was used to collect reticulocyte parameter results from patients at two clinical sites of Cincinnati Children’s Hospital Medical Center; consent was waived by the IRB. Samples were collected in K3-EDTA and stored at room temperature and analyzed within four hours. A minimum of 120 samples were used from four age groups (group 5 n=101), Group 1 = 1mo–2yr; 2 = 2-6yr; 3 = 6–12yr; 4 = 12-18yr. Exclusion criteria consisted of patients with abnormalities in RBC parameters (RBC, Hgb, RDW, indices), abnormal platelet count, patients with an ordered reticulocyte count, patients with hematologic, cardiac, and systemic inflammatory diseases, cancer, sepsis, ITP, status post-transplant, status post transfusion in the past six months, acute blood loss, and iron deficiency. Patients with ordered ESR, C-reactive protein, and serum measures of iron (serum iron, TIBC, ferritin) were excluded if any result was out of the reference range. The reference limits were calculated as per the CLSI C28-A3c published guidelines. Results: The reference intervals are in Table 1. Conclusions: References ranges for the extended reticulocyte parameters indicate age specific trends. Further studies are required to determine if these novel parameters improve the sensitivity and specificity of early detection of IDA in children.

Table 1: Pediatric Reference Intervals for Reticulocyte Parameters

<table>
<thead>
<tr>
<th>Group</th>
<th>MCVr (fl)</th>
<th>MCHR (pg/mL)</th>
<th>MCHCr (pg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>122</td>
<td>80.1–106.6</td>
<td>28.3–31.6</td>
</tr>
<tr>
<td>2</td>
<td>128</td>
<td>80.1–106.6</td>
<td>28.3–31.6</td>
</tr>
<tr>
<td>3</td>
<td>120</td>
<td>80.1–106.6</td>
<td>28.3–31.6</td>
</tr>
<tr>
<td>4</td>
<td>120</td>
<td>80.1–106.6</td>
<td>28.3–31.6</td>
</tr>
<tr>
<td>5</td>
<td>120</td>
<td>80.1–106.6</td>
<td>28.3–31.6</td>
</tr>
</tbody>
</table>

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PREVALENCE OF IRON DEFICIENCY AMONG MALE FREQUENT BLOOD DONORS IN CALABAR, CROSS RIVER STATE, NIGERIA
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Introduction: Iron deficiency is one of the most common nutritional disorders in the world, and blood donation may cause iron depletion (Javazdah et al., 2005). Methods: One hundred and eighty-four male blood donors comprising 5 groups namely; group 1, consisting of 35 first time donors (control), group 2, consisting of 32 second time donors, group 3, consisting of 35 third time donors, group 4, consisting of 41 fourth time donors and
group 5, consisting of 41 fifth time donors attending University of Calabar Teaching Hospital (UCTH) bleeding bay were used for the study. Blood samples were taken from all the donors and their iron-related parameters namely: haemoglobin concentration (Hb), transferrin saturation (TS), serum ferritin level (SF) and serum transferrin receptor level (STIR) were determined as indicators of iron stores. **Results:** The prevalence of anaemia 49 (26.6%), iron depletion 60 (32.6%), iron deficiency without anaemia 106 (57.6%) and iron deficiency anaemia 60 (32.6%) was significantly (P<0.05) higher in 184 male blood donors. It was observed to be higher in 3rd, 4th and 5th timers when compared with control (first) and second time groups. The higher prevalence of iron deficiency may be caused by more frequent and larger volumes of blood the 3rd, 4th and 5th timers donate when compared to control and second time groups. **Conclusions:** It is concluded that to avoid this differences in the prevalence among this donors, regular supervision of their haematocrit levels and introducing haemoglobin estimation (using autoanalyser) and serum ferritin be made for them before donating or else, 3rd timers and those above third should not be allowed to donate blood in a year. Moreover, adequate iron supplement should be given in order to prevent the development of iron deficiency.

**MONITORING RESPONSE IN IRON DEFICIENCY PATIENT USING MINDRAY BC-6800 & BC-3600 AUTOMATED HEMATOLOGY ANALYZERS**

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**Introduction:** Hypochromic microcytic anemia is a major health problem among developing countries especially in Southeast Asia. Among this group of anemia, iron deficiency anemia and thalassemia are the most common and may be distinguished by expensive and difficult techniques including viz. hemoglobin (Hb) typing, DNA analyses and iron status measurement. Finding cheaper and easy technique to differentially diagnose both diseases is important. New blood hematology analyzers BC-6800 has new parameters and properties that may be useful for this purpose.

**Methods:** Ten patients with hypochromic microcytic anemia confirmed to be due to iron deficiency, were recruited for measurement of red cell indices (RI) by automatic blood analyzer before and after daily oral iron supplements with 180 mg ferrous sulfate tablet. All blood specimens were collected using EDTA as anticoagulant. RIs were measured on Mindray BC-6800 and BC-3600 (Nanshan, Shenzhen, China). Patient’s iron status was measured by serum ferritin measurement. Hemoglobin analysis was done using an automated HPLC. Alpha- and beta-thalassemia genotypes were performed using GAP PCR and reverse dot blot, respectively. **Results:** Hb typing and DNA analyses confirmed absence of thalassemia genotypes in these ten hypochromic microcytic anemia cases. Low serum ferritin were detected and were used to confirm the diagnosis of iron deficiency in all patients. RI values including hemoglobin (Hb), hematocrit (Hct), MCV, MCH, MCHC & RDW of all cases showed similar elevation as the reticuloocytes values after iron supplement. The response could be observed as early as within 1 week after administration of iron supplementation. **Conclusions:** Our results demonstrated that follow-up of the changes in RI values obtained on BC-6800 & BC-3600 - following iron supplementation to treat hypochromic microcytic anemia cases - may be used as a good tool for therapeutic diagnosis in cases suspected to have iron deficiency anemia.

**VARIABILITY OF HEMOGLOBIN F EXPRESSION IN HEMOGLOBIN EE DISEASE: HEMATOLOGICAL AND MOLECULAR ANALYSIS**

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**Introduction:** Although the molecular basis of variability of Hb F has been extensively examined in b-thalassemia and sickle cell diseases, less study has been done on Hb E disorder. To address the variability of Hb F expression in Hb EE disease, we have examined multiple single nucleotide polymorphisms (SNPs) in b-globin gene cluster, BCL11A and HBS1L-MYB genes and determined their associations with Hb F levels in this syndrome. **Methods:** Study was done on 141 adult Thai individuals with homozygous Hb E. Hematological parameters were recorded and Hb F measured using Hb-HPLC analyzer. The a- and b-thalassemia mutations, ‘g-Xmn I polymorphism were examined by PCR and related techniques. SNPs in BCL11A and HBS1L-MYB genes were identified by PCR-RFLP and Real-time PCR. **Results:** It was found in 26 cases that co-inheritance of a-thalassemia could lead to significant lower production of Hb F. Association of Hb F expression with the ‘g-Xmn I polymorphism and other SNPs including rs2297339, rs2838513, rs4895441 and rs9399137 in HBS1L-MYB gene and rs4671393 and rs11886868 in BCL11A gene were therefore analyzed in the remaining 115 cases without a-thalassemia. It was found that 4 of these 7 SNPs including ‘g-Xmn I polymorphism (rs7482144), HBS1L-MYB (rs4895441) and (rs9399137) and BCL11A (rs4671393) were significantly associated with higher proportions of subjects with high Hb F (Hb F ≥ 5%). The result demonstrated that multiple genetic modifying factors are associated with increased Hb F and in combination could explain approximately 80% of the variation of Hb F in Hb EE disease in Thai population. **Conclusions:** This study demonstrated that genetic modifying factors including T allele of XmnI polymorphism (rs7482144), G allele of HBS1L-MYB (rs4895441), C allele of HBS1L-MYB (rs9399137) and C allele of BCL11A (rs4671393) are strongly associated with increased Hb F expression in Hb EE disease in Thai population. Additional genetic factors regulating Hb F expression in this common genetic disorder remains to be elucidated.
FINE-MAPPING OF TELOMERIC BREAKPOINTS OF CHROMOSOME 16P DELETIONS IN ALPHA-TALASSEmia CARRIERS AND ATR-16 PATIENTS

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Introduction: The majority of the alpha-thalassemia deletions involves one or both alpha-globin genes and can be detected by gap-PCR for the 7 most common deletions. However, larger deletions also occur in this region. When the deletion length exceeds beyond 1.5 Mb, they may be associated with a variable clinical picture, including alpha-thalassemia, dysmorphic features and mental retardation, which is known as the alpha-thalassemia/mental retardation syndrome (ATR-16). Most of these deletions either involve the complete telomeric end of chromosome 16p or leave the telomeric region intact. The aim of this study was to investigate different types of telomeric deletions on chromosome 16p, leading to alpha-thalassemia or ATR-16. By determining the breakpoint position of the deletions more precisely, it is possible to study the molecular mechanisms underlying these rearrangements. Furthermore, this knowledge enables design of relatively simple gap-PCR assays in some cases. Methods: A group of 25 patients was investigated, carrying a deletion on chromosome 16p detected by MLPA. Fine-tiling array CGH was applied to fine-map the deletion breakpoint more precisely. In cases with an intact telomeric region, primers were designed to perform gap-PCR and sequence analysis to determine the exact breakpoints. Results: From the 25 cases studied, the fine-tiling array CGH suggested an intact telomeric region in 13 cases. The other 12 patients carried deletions involving the telomeric end of chromosome 16p. Gap-PCR and sequence analysis to determine the exact breakpoint was successful in two cases. Conclusions: The arrayCGH results suggest two types of deletion mechanisms. The interstitial deletion with an intact telomeric region indicative of telomere capture as a mechanism and deletions involving the complete telomeric end, indicative of telomere healing. Furthermore, array CGH results enabled the design of gap-PCR assays for 2 deletions. Due to the repetitive nature of the telomeric sequence, it is very difficult to design unique primers in this region. However, determination of the 3’ end breakpoint by aCGH also provides valuable information. Loss of the 16p13.3 region may have significant clinical consequences such as the ATR-16 syndrome. It is yet unknown which genes are responsible for the mental retardation phenotype in these patients. Therefore, it is important to study this kind of deletions to gain more insight in the genotype-phenotype correlations.

HEREDITARY SPHEROCYTOSIS SCREENING IN CHILDREN USING THE CELL-DYN SAPPHIRE HEMATOLOGY ANALYZER

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1Our Lady’s Children’s Hospital, Crumlin Dublin, Ireland, 2Abbott Diagnostics Division Wiesbaden-Delkenheim, Germany

Introduction: Hereditary spherocytosis (HS) is the most common haemolytic anaemia in north-western Europe. The diagnosis is based on a combination of family history, clinical findings and laboratory data, including the presence of spherocytes. The osmotic fragility test is used for diagnostics, but is not suited for screening. Spherocytes have increased cellular haemoglobin concentration, but MCHC is an insensitive parameter for detecting spherocytes. Some haematology analysers can measure hyperchromic erythrocytes (HPR), using multidimensional optical scatter of spheroid RBC and applying the Mie theory. Since spherocytes are hyperchromic, HPR might be useful for HS screening. The aim of this study was to assess the utility of HPR as a screening tool for HS. Methods: We analysed blood from 740 paediatric patients between 0 and 16 years of age with a variety of diseases, in the reticuloocyte mode of the CELL-DYN Sapphire haematology analyser (Abbott Diagnostics, Santa Clara, CA, USA) for obtaining HPR. In all samples the flow cytometric EMA test (eosin-5’-maleimide staining of erythrocyte cytoskeleton proteins) was performed as the reference method. For data analysis we used standard statistical methods. Results: Stability studies with blood from HS patients showed a significant decrease of HPR after 6 hours. Therefore, samples were measured within 6 hours from blood collection. HPR results in the most relevant patient groups are shown in the Table. In the vast majority of healthy children, no HPR were found: few children had detectable HPR, but never more than 1.0%. In the HS group HPR were significantly higher than in all other groups (P < 0.001, Mann-Whitney test). There was only a single AIHA patient whose HPR overlapped with the HS group. Patients with other diseases had always < 5.0% HPR. In receiver-operator-characteristics analysis we found a very high area under the ROC curve: 0.972 (95% confidence interval 0.957-0.983). When using 4.9% HPR as a cut-off, the sensitivity of detecting HS was 96.4% and the specificity was 99.1%.

Conclusions: Hyperchromic erythrocytes as measured on CELL-DYN Sapphire represent a very sensitive and specific test for detecting spherocytes. HPR can be used as a rapid and inexpensive screening test for hereditary spherocytosis.

ANALYTICAL VALIDATION OF THE RED BLOOD CELL AGGREGATION TEST AND THE ERYTHROCYTE DEFORMABILITY TEST ON THE LORRCA MAXISIS®

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Alkmaar Medical Center Alkmaar, Netherlands

Introduction: With the LoRRca MaxSIS analyzer it is possible to measure red blood cell (RBC) properties. The RBC-aggregation syllectogram distinguishes four behavioral stages: disaggregation, RBC-shape recovery, rouleaux-formation and 3D-aggregate formation. Results are expressed in extend of aggregation (amplitude), kinetics of aggregation (T1/2), aggregation index (AI) and threshold shear rate (Y at dIsc min). The RBC-deformability curve indicates the change in the diffraction pattern during application of increased shear stress, expressed in the maximum
elongation index (EL_{max}) and the pressure needed to elongate a cell for half of its maximum elongation (SS1/2). Methods: Performance characteristics of the erythrocyte deformability and aggregation tests were evaluated. K2EDTA anticoagulated blood samples were analyzed within 4h after collection. Intra- and inter-assay variation was determined in 10 samples in 3-fold. Sample stability was performed up to 48 hours with 4 samples at room temperature. Results: Intra- and inter-assay variation were AI <3%, amplitude <5%, T1/2 <7% and y disc min <10%. The effect of time up to 24 hours did not obviously affect RBC aggregation results; afterwards a decrease of 25% in amplitude was detected. Results concerning intra- and inter-assay variation yielded appropriate results for ELmax <1% and SS1/2 <6%. The effect of time up to 48 hours did not obviously affect RBC-deformability results. Conclusions: RBC aggregation and erythrocyte deformability assays on the LoRRCa MaxSIS have a good analytical performance. Results of the current evaluation are promising for additional investigations in clinical settings.

737 GASTROINTESTINAL ABNORMALITY IN THALASSEMAIA MAYOR CHILDREN WITH IRON OVERLOAD INA S TIMAN, AGUS FIRMANSYAH, SITI B KRESNO, DIANA AULIA, MOESLICHAN MZ FACULTY OF MEDICINE UNIVERISITY OF INDONESIA Jakarta, 13340, Indonesia

Introduction: In Indonesia, thalassemia major children with multiple transfusion have severe iron overload. Currently, no study has been conducted to evaluate the overall effect of iron overload to the gastrointestinal. The aim of this study is to know the effect of iron overload on the pancreas, gastro, enterocyte and liver. Methods: A cross sectional study was conducted in the University of Indonesia, Eijkman Institute Jakarta and Utrecht. The subjects consisted of 108 thalassemia patients with iron overload and 86 without iron overload. Pancreas was evaluated using elagastase E-1, enter was evaluated with pepsinogen II, intestinal function was evaluated as lactase/lactulose activity, Fecal α1-antitrypsin (FAAT) for protein-loosing enteropathy, liver was evaluated using collagen IV. Correlations were analyzed to NTBI, transferrin saturation and ferritin. Results: The iron overload group consisted of 108 subjects aged 1.2 – 23 years (mean 8.1) and the non iron overload group consisted of 86 subjects age 2 – 49 years old (median 32) as control. In the thalassemia with iron overload, 52.8% (57/108) has low body height, 34.3% (37/108) low body weight and 5.6% (6/108) were undernourished. The levels of Pepsinogen II in iron overload subjects were 10.35 ug/L, significantly lower compared to controls (p < 0.05), which showed impairment of the stomach function. In comparison to the healthy control, significant lower lactase activity and impairment of intestinal integrity was observed (p < 0.05). There is negative correlation between transferrin saturation to elastase E-1 (r = -0.478; p = 0.000), and between NTBI to elastase E-1 pankreas (r = -0.427; p = 0.000). Only weak correlation was found for lactulose excretion and transferrin saturation (r = -0.189; p = 0.018). The iron overload group showed higher protein loss as shown by the FAAT compared to control. The collagen IV value in iron overload subjects were 124.6ng/mL, significantly higher (p<0.05) compared to controls, 101.6ng/mL. Moderate correlation were found between collagen IV to ferritin (r=0.572, p=0.000) and NTBI (r=0.355, p= 0.001). Conclusions: Abnormalities in iron overload subjects were found in all organs, stomach, liver, pancreas and intestine. Those abnormality might contribute impairment of digestion and absorption of nutrients as shown by the low height and weight of the thalassemia children. Following this study, it is recommended that clinical study should be performed on the benefit of pancreatic and small intestines enzymes supplementation to improve the growth in thalassemic children.

739 USE OF SERUM FERRITIN IN PREVENTING SECONDARY HEMOCHROMATOSIS AFTER TRANSFUSION IN A REGULAR HOSPITAL SETTING Gita van den Berg1, Jarno Bouwes2, Jan W Smit2, Pamela MJ McLaughlin2,1
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Introduction: Polytransfused patients used to comprise of hemoglobinfile patients, yet now a days, the largest cohort of polytransfused patients in a regular hospital setting are patients treated for solid or hematologic malignancies. As with hemoglobinopathies the risk of iron overload is present. Since one packed RBC holds about 250mg Fe and one can only get rid of 1-2mg iron per day, iron overload is likely to occur. But at what amount of blood is this threat real? Monitoring serum ferritin levels has been described useful in preventing organ damage by secondary hemochromatosis. To establish if serum ferritin levels could be used to monitor our cohort of polytransfused patients we conducted a retrospective data analysis. Methods: Ferritin was measured on the Roche Modular E-module. Patient data were analysed and patients selected based on 20 or more packed RBCs transfused for tumor treatment purposes over the last 5 years with at least one ferritin level over 1000microgram/l. Statistical analysis were performed using Analyse it (Microsoft). Results: A total of 237 patients obtained over 20 packed RBCs over the last 5y. Of these 53 never had a ferritin level exceeding 1000 microgram/l, while 184 did. Of the later, 52 patients were transfused in relation to tumor treatment. When analysing individual ferritin levels in relation to cumulative transfusions a correlation seemed present (fig 1), however, when analysing the mean ferritin level per amount RBCs transfused no correlation was established. The mean ferritin level fluctuated at 2000microgram/l, irrespective of the amount of packed RBCs administered. Correcting for the number of packed RBCs per time-interval (3months) did not change this outcome. Conclusions: After cumulative transfusions an increase in serum ferritin levels reflecting tissue iron-content is to be expected. Although confirmed on individual patient level, no correlation with the number of packed RBCs transfused and serum ferritin level could be established. However, already at 20 packed RBCs the mean ferritin level rose to the critical level of 2000microgram/l, a level described to be associated with negative-effects of iron-overload. Therefore we recommend follow-up of ferritin levels in patients transfused with 20 or more packed RBCs in the context of anti-tumor treatment if more transfusions are forseen.
Comparing of MCH and MCV in children with iron deficiency and mild & beta;-thalassemia

Nadezda Vavynuskaya, Natalia Sokolova

Introduction: Differential diagnosis of hypochromic anemias begins with the blood test. The most difficult problem for ambulant hematologist is the suspect the disease and to administrate additional needed analysis when he sees the result of blood test the first time. Methods: 40 children from 6 months of age till 18 years (first group) with proven iron deficiency mild anemia and 40 children the same age with proven beta thalassemia minor (second group) made blood test on the hematology analyzer ADVIA 2120. Results: The level of Hb in both groups of patients ranged from 90-108 g/l, the mean Hb was 101, 22g/l. MCH of patients in first group ranged from 21, 5 - 24,7fl, the average was 23, 47fl and MCV ranged 63 - 73 pg, the average was 69 2pg. The patients of the second group had MCH ranged from 16,3 to 19,9fl, 18,37 was the average. MCV was 55-63pg, the average was 58,7pg. Conclusions: In children with minor form B-thalassemia indicators mean corpuscular volume and mean content of Hb in the erythrocyte is significantly lower than in patients with mild iron deficiency anemia. That allows doctor to suspect the presence of hemoglobinopathies and not to prescribe ferrotherapy even in younger children, also it can help to administer hemoglobin electrophoresis test early. Making right choice of needed analysis is very important for developing country where policlinics can not afford doing all tests because of economic reasons.

The Percentage of Hyperchromic Red Blood Cells (%HYPER) Discriminates Between HEMOGLOBIN S TRAIT AND HEMOGLOBIN C TRAIT

Diego Velasco-Rodriguez, Juan Manuel Alonso-Dominguez, Fernando Ataulifo Gonzalez-Fernandez, Paloma Ropero, Natalia Acedo, Cristina Seri, Raquel Guillen, Beatriz Alvarez, Jesus Villarrubia

Introduction: The haemoglobinopathies are the most common genetic disorders worldwide. Due to the current immigration trends, their prevalence in our country has increased. Hemoglobin S trait (HbS) and hemoglobin C trait (HbC) are the most common structural haemoglobinopathies in our geographic area. The erythrogram provided by Advia 2120 analyzer (Siemens) classifies red blood cells (RBC) in 9 subpopulations according to their MCV (macro, normo and microcytic) and their MCHC (hyper, normo and hypochromic). Methods: All prospectively diagnosed HbS and HbC cases (based on alkaline and acid electrophoretic pattern) in our laboratory between October 2012 and April 2013 were evaluated. According to published studies, subjects with percentage of variant Hb [determined by HPLC in HA-8160 analyzer (A. Menarini Diagnostics®)] <35% (for HbS) or <37% (for HbC) were considered to have coexistent alpha thalassaemia. RBC subpopulations provided by Advia 2120 (%MACRO, %MICRO, %HYPER, %HYPO) were assessed. Independent sample t-test was used to compare RBC subpopulations between HbS, HbC and healthy controls, and receiver operating characteristic (ROC) curves were plotted.

Results: Patients: 164 variant Hb (124 HbS and 40 HbC) and 128 normal controls. According to our criteria, 73 HbS (58.87%) and 9 HbC (22.5%) had associated alpha thalassaemia. Statistically significant differences were found between normal controls and variant hemoglobins in: %MACRO (0.72% vs 0.39%, p < 0.001), %MICRO (0.53% vs 4.86%, p < 0.001), %HYPER (0.73% vs 1.11%, p = 0.014), %HYPO (1.23% vs 4.72%, p < 0.001). A higher %HYPER (2.75% vs 0.58%, p = 0.014) and lower %HYPO (1.7% vs 5.7%, p = 0.002) were found in HbC in comparison with HbS. No differences in %MACRO and %MICRO were found. ROC analysis revealed that %MICRO was the best parameter to discriminate between normal controls and variant Hb (AUC 0.871), and the cut-off with the best combination of sensitivity (SE) and specificity (SP) was 0.65% (SE = 82%, SP = 75%), %HYPER was the most efficient to distinguish HbC from HbS (AUC 0.874), and the best cut-off was 0.95 (SE = 80%, SP = 79.8%). Conclusions: RBC subpopulations provided by Advia 2120 discriminate patients with and without variant Hb. Haemoglobinopathy investigation should be performed in subjects belonging to ethnic groups with high prevalence of variant Hb if %MICRO is >0.65%. If a patient with a variant Hb not yet identified shows %HYPER >0.95% the diagnosis is more likely to be HbC. This reflects the higher dehydration of RBC in HbC. Further studies are needed to confirm these results.

Demographic Impact in Madrid of Variant Hemoglobins Detected during Glycosilated Hemoglobin Measurement

Diego Velasco-Rodriguez, Juan Manuel Alonso-Dominguez, Fernando Ataulifo Gonzalez-Fernandez, Paloma Ropero, Natalia Acedo, Cristina Seri, Raquel Guillen, Beatriz Alvarez, Jesus Villarrubia, Fernando Cava

Introduction: The haemoglobinopathies are the most common genetic disorders worldwide. Due to the current immigration trends, their prevalence in our country has increased. Several studies have been conducted in Spain, with reported incidences from 1.25 to 1.47 per 1000 person-years. Measurement of glycosilated hemoglobin (HbA1c) is widely used for routine monitoring of glucose in patients with diabetes. During determination of HbA1c, samples with variant Hb can be identified. Samples from 5 geographical areas of Madrid are processed in our laboratory: Vallecas and Arganda del Rey (area 1), Coslada (area 2), San Sebastian de los Reyes (area 5), Parla (area 10) and Aranjuez (area 11).

Methods: Variant hemoglobins incidentally found during HbA1c testing by HPLC [HA-8160 analyzer (A. Menarini Diagnostic®)] in our laboratory were prospectively recruited from October 2012 to April 2013. Hb variants were identified according to their alkaline and acid electrophoretic pattern. Sequencing of the beta (or alpha) globin gene was performed in those with inconclusive pattern. Data of all HbA1c measurements in that period of time were collected.

Results: HbA1c was measured in 80819 patients, 159 of which presented variant Hb (estimated incidence 1.96 per 1000 person-years). Type of variant Hb: 109 HbS trait (HbS) (68.55%), 35 Hb C trait (HbC) (22.01%), 6 Hb D trait (3.77%), 3 Hb S homozygous (1.88%), 3 Hb O-Arab (1.88%), 1 Hb SC (0.63%), 1 Hb E trait (0.63%), 1 Hb G Philadelphia (0.63%). The estimated incidence for the different areas was: 1.56 per 1000 person-years in area 1, 0.54 per 1000 person-years in area 2, 2.03 per 1000 person-years in area 5, 4.11 per 1000 person-years in area 10 and 2.16 per 1000 person-years.
years in area 11. The distribution of newly diagnosed Hb variants depending on the different areas of Madrid is summarized in Table 1. Conclusions: Identification of variant Hb carriers is clinically important, since genetic counselling can prevent the occurrence of homozygous and compound heterozygous subjects. This strategy can be cost-effective, thus reviewing HbA1c studies to detect variant Hb is recommendable. A higher incidence of variant Hb in Madrid was seen in our study in comparison with previous Spanish studies. The highest incidence was seen in Parla (area 10) which is the city with the highest percentage (26%) of immigrants in Madrid area. In our experience, 2 out of 3 newly diagnosed Hb variants are HbS, being HbC the second most common.

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Table 1. Incidence and types of variant haemoglobin according to geographical area.

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747 GENOTYPE AND PHENOTYPE DIVERSITY OF THALASSEMIA AMONG POSITIVE-SCREENED LAOTIAN COUPLES
Kasama Wongprachum1,2, Kanokwan Sanchaisuriya3, Maneely Dethvongphanh1, Boulay Norcharoen1, Bousanit Htalongsengchan1, Virak Vidamaly1, Pattara Sanchaisuriya4, Supan Fucharoen2, Goonnapa Fucharoen2, Frank P. Schelp4
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Introduction: A pilot screening program for thalassemia has been initiated at Maria Teresa Hospital (MTH), Lao PDR, since 2012. This study aimed to describe the diversity of genotype and phenotype of thalassemia among the positive-screened Laotian pregnant women. Methods: Laotian pregnant women with gestational age less than 16 weeks and their partners were invited to join the program. The mean corpuscular volume (MCV) and the mean corpuscular hemoglobin (MCH) were determined using capillary zone electrophoresis (CZE) and the DCIP test were used as screening tool for thalassemia. Hemoglobin (Hb) profiles were determined using capillary zone electrophoresis (CZE). PCR-based technology was applied to identify alpha- and beta-thalassemia mutations. Results: From March 2012 to December 2013, 411 pregnant women were screened. Of these, 224 (50.8%) were positive, and 71 (31.7%) partners of the positive-screened women participated. Amongst the positive-screened couples, 20 different genotypes were identified. Heterozygous Hb E was most common (20.7%), followed by heterozygous alpha-(0)-thalassemia (19.8%), heterozygous alpha-(-)-thalassemia (9.9%), and heterozygous beta-thalassemia (3.6%). Many complex thalassemia syndromes including 8.5% non-transfusion dependent thalassemias (NTDT), i.e. Hb E-beta-thalassemia, Hb H, and EABart’s diseases, were found. Severity of anemia among the NTDT varied considerably with Hb range from 5.3 to 9.2 g/dL. Of the NTDT, an unusual phenotype of Hb E-beta-thalassemia with 89.1% Hb E, 1.6% Hb F and 9.3% Hb A, was identified. Hematologic analysis of her blood showed moderate anemia (Hb 7.7 g/dL) with a marked reduction in MCV (45.0 fl) and MCH (12.6 pg). Further analysis of alpha- and beta-thalassemia mutations revealed the actual genotype of Hb E-beta-(0)-thalassemia (codons 41/42, -TTCT mutation) co-inherited with alpha-(0)-thal (SEA deletion). As her husband was Hb E heterozygote, the fetus of this couple was therefore at-risk for Hb-E-beta-thalassemia disease. Conclusions: The findings indicate a remarkable diversity of genotype and phenotype of thalassemia among Laotian population. To provide appropriate genetic counseling and care, laboratory results need to be interpreted carefully.

749 CALCULATION OF MULTICENTRE REFERENCE INTERVALS FOR HAEMATOLOGY PARAMETERS ON SYSMEX XN SERIES analysers
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Introduction: In line with different regulations, clinical laboratories have to check their reference intervals in use. Since the procedure to calculate reference intervals is time-consuming and costly and reference individuals are not always available, a lot of laboratories transfer published reference intervals. Alternatively, reference intervals can be calculated in a multicentre setting. In this study, we calculated multicentre reference intervals for haematology counts on the Sysmex XN haematology analysers. For this new analyser series, no specific reference limits have been published yet. Methods: Ten hospital laboratories, located in a narrow geographical region (Flanders, Belgium) and serving a similar population, participated in this multicentre study. All laboratories were using an XN haematology analyser (Sysmex), according manufacturer’s instructions. Quality assurance, including national proficiency testing, of every instrument was monitored according to local laboratory practice. Each laboratory enrolled and analysed a group of reference individuals (n=20 to 57). CBC results, including white blood cell differential and reticulocytes, of all reference individuals of all 10 laboratories were collected and pooled. Non-parametric reference intervals (2.5-97.5%) were calculated according to CLSI guidelines. Partitioning of data according to gender was done in line with published recommendations. Results: Three hundred reference individuals (female 200; male 100; median age: 41 yrs, range 20 - 60 yrs) were included. Separate reference limits for females (F) and males (M) were calculated for haemoglobin (F: 11.8-15.5, M: 13.7 – 17.1 g/dL), haematocrit (F: 35.5 – 46.5, M: 40 – 51.7 %) and red blood cell count (F: 3.75 – 5.11, M: 4.30 – 5.71 10E12/L). Reference ranges for red cell indices included MCV (84.0 -98.3 fL), MCH (27.6 – 32.9 pg) and MCHC (31.6 – 35.1 g/dL). Other reference limits included white blood cells (4.2 – 9.8 10E9/L), platelets (162 – 351 10E9/L) reticulocytes (6.4 – 19.9/1000rbc) and 6-part differential: neutrophils (39.6 – 81.0 %), lymphocytes (13.0 – 43.0 %), monocytes (3.0 – 10.0 %), eosinophils (0.7 – 8.0 %), basophils (0.2 – 0.8 %) and thrombocytes (200 – 400 10E9/L).

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HEMATOLOGIST AND CLINICIANS IN ITALY: THE SIMeL QUESTIONNAIRE REPORT

Bruno Biasioli, Anna Maria Cenci, Piero Cappelletti, Valentino Miconi, Mauro Buttarello, Luciano Pasini, Maria Golato, Marco Moretti

Hematology Working Group (GdS-E) of Italian Society of Laboratory Medicine (SIMeL) Castelfranco Veneto, Italy

Introduction: In 2012-13 SIMeL GdS-E investigated the organization of the laboratory hematology in Italy by a survey submitted to laboratory professionals. Methods: 148 colleagues replied to a 24-items questionnaire, providing informations about organization and managing of the hematological diagnostic workflow, test types, CBC validation rules, criteria and impact of the microscopic review, characteristics of the communication with the clinical users. Results: The rules for microscopic review are shared with the clinicians in 20/148 (13.5 %); the criteria for reporting (comments) in 14/148 (9.45%). The communication with the clinical wards is mainly based (100/148, 67.5%) on webreport delivery: in the hospital (49/100, 50%), to the general practitioners (8/100; 8%) or both (32/100, 32%). Telephone contact is used (93/148, 62.8%) as well as other different facilities (36/148, 24%) such as e-mail (10, 27.7%), fax (10, 27.7%) and personal contact (4, 11%). A provided assessment and control of the service (audit or feedback) is declared by 44/148, representing 29.7%. Conclusions: Accreditation rules, aimed to standardize and consolidate communication processes, appear largely disregarded. Mainly, criteria for review and comments declared as standardized are coded only in medium and big size hospitals and in the northeast area of Italy; their sharing with clinicians and the implementation of feedback/audit activities are really low (less than 30%) and occurs in north-eastern Italy again. The clinical-laboratory interface relationship seems to be limited to include comments and diagnostic suggestions onto report, mostly without proactive attitude; while in communication the growing utilization of tools such as e-mail or phone to communicate, can be interpreted as an encouraging sign, allowing a more “friendly” contact. All these features lead to consider the task of the Scientific Societies and its Working Groups in promoting these fundamental aspects of Laboratory Medicine, in order to manage the clinical-laboratory relationship and its potential value properly.

HEMATOLOGY LABORATORY TRAINING NEEDS IN ITALY. THE GDS-E SIMeL 2012-13 QUESTIONNAIRE

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Hematology Working Group (GdS-E) of Italian Society of Laboratory Medicine (SIMeL) Castelfranco Veneto, Italy

Introduction: GdS-E SIMeL intended to know the training needs of professionals managing laboratory hematology in Italy. A recent survey tried to investigate this topic. Methods: In 2012-13 a questionnaire of 24 different items has been submitted to a large number of professionals, 169 of them answered choosing a maximum of 3 items within a list of preset training needs. Results: The expressed training needs concerned: morphology (43.8%), hemoglobinopathies (39%), management (29.6%), clinical items (27.8 %), biochemistry (27.2 %), flow-cytometry (25.4 %), cytometry (17.1 %), genetics (7.7 %), cytochemistry (4.1 %), cytogenticities (3.5 %). Considering years of career of the participants, they were shared in three groups (<15 y, 15-25 y, >25 y) with similar results about the overmentioned items. In particular, <15 y group was interested in genetics, while >25 y group had no particular requires about cytochemistry. Linking geographical groups and items, the northeast Italy ones declared needs concerning, in order, cytometry, morphology, biochemistry,
genes and organization; central-south Italy groups asked for managing items, excluding hemoglobinopathies diagnosis. In laboratories of hospitals <200-beds sized interest is focused on biochemistry and clinical-items, in hospitals 200-800-beds sized to morphology and flow cytometry and in those >800-beds sized to cytometry. **Conclusions:** This survey demonstrated that training in morphology does not appear useful as in the past was (55% GdS E 2005); the importance of the hemoglobinopathies screening is confirmed (45%). The interests for management, clinical items, biochemistry (serum proteins diagnostic included) and flow-cytometry are confirmed, while cytometry, cytogenetics, genetics and cytochemistry are not required (<10 %,≈2005). Growing interest is expressed in the relationship with the Clinicians and in understanding the workflow management. Professionals career length can produce different needs. In conclusion, the traditional educational target must apply greater attention to the above-mentioned themes. Bone-narrow diagnostic involves a small but standing number of laboratories; cytochemistry constitutes an educational objective if scheduled into specific diagnostic pathways. Lack of interest for cytometry, most in young people, probably means widespread knowledge, but also “instrumental confidence” without critical knowledge. According to what above expressed, GdS-E needs to set new training activities with a wider and customer oriented choice of training opportunities.

**807**

**THE RSD \( x_{50}\% \) AND 75% _A_ RULE COMBINATION CAN EFFICIENTLY INTERPRET PROFICIENCY TESTING (PT) DATA PRODUCED BY HIGHLY PRECISE HEMATOLOGY ANALYZERS**

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**Introduction:** In the absence of large analytic errors that exceed regulatory limits, the inspection of PT results for potentially important biases or increased random error can be subjective. Carey et al suggest that three simple rules can identify significant analytic error in sets of 3-5 proficiency test results, one rule to demonstrate increased random error, another to demonstrate analytic bias and the other to demonstrate the excursion of one or more of the results that are dangerously close to the regulatory limits (termed a near-miss). For PT results generated by highly precise analyzers, increased random error is demonstrated by violation of the RSD (a range rule) which requires the difference between the largest and smallest PT result to exceed 4 SDI [Standard Deviation Index]. A significant bias is defined by the mean rule, x 1 SDI, which requires the average of the PT results to exceed 1.5 SDI. Finally the 1-75% _A_ is invoked when at least 1 result exceeds 75% of regulatory analyte-specific allowable limits (termed a near-miss). For PT results generated by highly precise analyzers, increased random error is demonstrated by violation of the RSD (a range rule) which requires the difference between the largest and smallest PT result to exceed 4 SDI [Standard Deviation Index]. A significant bias is defined by the mean rule, x 1 SDI, which requires the average of the PT results to exceed 1.5 SDI. Finally the 1-75% _A_ is invoked when at least 1 result exceeds 75% of regulatory analyte-specific allowable limits (termed a near-miss). This rule combination works well with high sigma analyzers; it signals few false rejections with small analytic errors and a high prevalence of true rejections with analytic shifts of 2-3 s magnitudes. **Methods:** We retrospectively applied the 3 rules to 8 consecutive reports (2011-2013) of College of American Pathologists PT results for 2 Sysmex XE-2100 analyzers (Sysmex America, Lincolnshire IL) operated at VGH. Each flagged potential analytic error was investigated by examining the quality control results from the same period. **Results:** There were no RSD violations but 6 x 1 SDI violations (3 MCV shifts associated with a single hematoctrit shift and a %monocytes and a single absolute monocytes shift). Outlying quality control results were identified for two of three MCV shifts and the hematoctrit shift. There were multiple violations of the near-miss rule (7 for %lymphocytes, 2 %eosinophils, 3 each of %basophils and %neutrophils, and 1 %monocytes). **Conclusions:** The absence of random error violations is consistent with the Sysmex XE-2100 being a high sigma analyzer. That 3 of the 6 shifts were correlated to shifted QC data reinforces the rules’ usefulness. The frequent near-miss violations indicate the need for only reporting absolute differential counts, rather than relative %counts.

**809**

**DISCREPANCY IN HEMOGLOBIN RESULTS BETWEEN AUTOMATED ANALYZERS CAUSED BY DIFFERENCES IN HEMOGLOBIN STANDARDIZATION.**

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**Introduction:** The measurement of the hemoglobin concentration in blood is one of the basic laboratory methods and is daily performed in clinical laboratories worldwide. For the hemoglobin concentration an International Council for Standardization in Hematology (ICSH) reference method and standard are available based on the measurement of hemoglobin cyanide (HiCN). We observed a consistent discrepancy between the hemoglobin concentration measured on an Abbott Cell-Dyn Sapphire hematology analyzer versus a Siemens Rapidlab 1265 bloodgas analyzer despite rigorous quality control and calibration measures. **Methods:** Hemoglobin concentration was measured on both analyzers in 40 randomly selected venous EDTA blood samples over a wide hemoglobin concentration range. **Results:** Using linear regression analysis we found a consistent 4.8% higher result in the bloodgas analyzer versus the hematology analyzer (Cell-Dyn Sapphire = 1.048 x Rapidlab 1265). This discrepancy is most likely due to a difference in standardization of both types of analyzers of the molecular extinction of HiCN at 540nm of the reference method. The Abbott Cell-Dyn Sapphire using an extinction coefficient of 11.0 L/mmol/cm derived from the ICSH reference method and standard; and the Siemens Rapidlab 1265 using 11.5 L/mmol/cm as HiCN reference derived from the first determinations of the HiCN molecular extinction by Drabkin et al. **Conclusions:** Differences in hemoglobin concentration reported by these 2 automated analyzers are likely due to the use of 2 different molecular extinctions for HiCN, leading to an overestimation of the hemoglobin concentration by the Rapidlab 1265 bloodgas analyzer.

**811**

**TOWARD A REFERENCE METHOD FOR MORE ACCURATE PLATELET COUNTS IN BLOOD:**

**COMPARISON OF PROPOSAL FLOW CYTOMETRIC METHODS WITH THE REFERENCE METHODS SPECIFIED TO THE CLSI H26-A2 STANDARD**

Yuki Jishage1, Yutaka Nagai1,2, Shigekyo Yamamoto1, Kazunori Chiba1, Etsuko Ogawa1, Hiroshi Kondo2
1IVD Technology Center, Nihon Kohden Corporation Tokyo, Japan, 2Department of Health Science, Daito Bunka University Saitama, Japan, 3BD Biosciences Tokyo, Japan

**Introduction:** The reference method for platelet counting was clearly specified in the CLSI H26-A2 standard as a flowcytometric method (FCM). The accuracy needs to show that the results are guaranteed by the value obtained using a reference method (ICSH/ISLH 2001). The necessary considerations for the better management of platelet counting were assumed to be: obtaining a direct counting value without use of a hematology analyzer; establishing a lower limit threshold of platelet size to prevent...
counting errors due to platelet-derived microparticle (PDMP) defined by International Society on Thrombosis and Haemostasis (ISTH); ensuring stability after preparation of test samples; and shortening the measuring time for sample with low concentration platelet. We studied a proposal reference FCM method with use of standard operating procedure (SOP) including those considerations. We validated both SOP and compared the proposed reference method with the manual microscopic method and ICSH/ISLH method. Methods: The manual reference method defined by WHO-ICSH method in 2000 was used. The FCM reference method defined by ICSH-ISLH in 2001 was used. Antibody to identify for platelets was used CD61 and CD41. The reagents were prepared by each proposed SOP. Beads (0.5µm, 1.0µm, 2.0µm, 1.56µm) were used for setting FCM. Paraformaldehyde (1%) was used for reducing the influence of natural activation of the platelet. For FCM platelet counting, BD Trucount tubes were used to determine the absolute numbers of the cell populations in addition to their percentages. Blood samples - To validate imprecision and accuracy for the SOP, fresh whole blood anti-coagulated with K, EDTA was collected from 52 healthy volunteers. Measurement - Flow cytometric analysis was performed using FACs Canto II (BD Biosciences) in validation of the SOP. Statistics Analysis - Excel, StatisPro (CLSI) and MedCalc (Passing-Bablok regression, Bland-Altman plot) Results: The imprecisions were CV% 1.9-2.4% (237-416/L). The comparison results of slope and intercept (1.032 and 0.375) were within the 95% confidence intervals of the CLSI H26-A2. Conclusions: Proposed reference method including the SOP was validated as a reference method for platelet FCM counts in blood for evaluation of automated hematology analyzers.

813 NOT TO MIX K2EDTA VACUUM TUBES AS RECOMMENDED BY ALL MANUFACTURERS: IS IT A NON CONFORMITY FOR QUALITY SYSTEM? - PRELIMINARY EVALUATION
Gabriel Lima-Oliveira1,2, Giuseppe Lippi1, Gian Luca Salvagno1, Giorgio Brocco2, Stefania Gaino1, Francesco Dim01, Waldemar Volanski2, Fabiane Gomes Rego2, Geraldo Picheth2, Gian Cesare Guidi2
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Introduction: Correct mixing after blood collection is claimed to be important and recommended by all manufacturers (e.g. gently and carefully by inverting 5 times). Presently laboratory quality managers has shown that phlebotomists do not mix vacuum tubes as recommended by manufacturers. Regards ISO 15189 international standard, necessary improvements and potential sources of nonconformities, either technical or concerning the quality management system shall be identified and all laboratory process shall be validated. The aim of this study was to evaluate whether it is really necessary to mix K2EDTA vacuum tubes immediately after blood collection. Methods: Blood collection: samples from 100 volunteers were draw in three 3.0mL vacuum tubes containing 5.9mg K,EDTA (Terumo Europe, Leuven, Belgium). To eliminate any potential interference due to either the contact phase or the tissue factor, ~2mL of blood were preliminarily collected in a discard tube without additive. Blood collection was accurately standardized, including the use of needles and vacuum tubes of the same lot. Processing: All vacuum tubes were processed using 3 different methods. Method 1: Gold Standard (M1): All specimens were mixed gently and carefully by inverting 5 times as recommended; Method 2: Rest time (M2): All specimens remained 5 min in upright position, followed by gentle careful mixing by inverting 5 times; Method 3: No mix (M3): All specimens were left in upright position without mixing afterwards. The influence of the primary tube mixing procedure was evaluated for routine hematology testing by paired t-test. Laboratory testing: All samples were processed for routine hematological testing immediately after collection (<15 min) on the same Sysmex® XE-2100D, Automated Hematology Analyzer (Sysmex Corporation®, Kobe, Japan). The parameters tested included erythrocytes, hemoglobin, hematocrit, mean corpuscular volume, RBC distribution width, reticulocytes, white blood cells count and differential, including neutrophils, lymphocytes, monocytes, eosinophils and basophils, platelet count and mean platelet volume. The instrument had been previously calibrated against appropriate proprietary reference standard material and verified with the use of proprietary controls. Results: No fibrin filaments or microclots were observed in any samples. Significant differences(P<0.01), were found only for: a) erythrocytes(0.5%) and haematocrit(1.1%) when M1 was compared with M2; b) erythrocytes(-0.9%) and haematocrit(-0.8%) when M2 was compared with M3. Conclusions: This preliminary evaluation has shown that K2EDTA tubes mixing after collection with evacuated system appears to be unnecessary. Furthermore this outcome suggest that do not mix K2EDTA vacuum tubes as recommended by manufacturers is not a non conformity for quality system.

815 ISO15189:2012, UNCERTAINTY OF MEASUREMENT AND LABORATORY HAEMATOLOGY
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Introduction: Lack of consensus of the most appropriate statistical methods for quantification of Uncertainty of Measurement has led to confusion. Those used are rarely appropriate for the task being performed. An analysis of routine and specialised haematology assays shows the analyte being measured and the means by which uncertainty is assessed has a significant bearing on perceived confidence of results provided by haematology laboratories. Methods: Uncertainty of Measurement was assessed using statistical analysis of Internal QC from January 2103 to January 2014 – the Top-down method Results: Uncertainty of measurement due to imprecision bias in assays measuring a single analyte directly such Hb, WBCC and Platelet count range from 2 to 2.7% compared to 7-8% for PT and aPTT and around 10% for Factor VIII, Protein C and Antithrombin. There is no significant difference between analysers for these assays. Uncertainty is increased further when imprecision due to commercial calibration is considered and increases to as high as 20% for Factor VIII. Number of data points used for analysis influences the uncertainty. Changes in reagent lot number also influences uncertainty and suggest assessment should be carried out following both reagent and QC material lots changes. Factor deficient plasma across four lot number changes, gave mean (6-7%) and SD (1%) variation for the same IQC material. Consequently, imprecision bias was affected by about 1%. Conclusions: Results presented here highlight the difficulty associated with determining and accurately quantifying uncertainty of measurement in the haematology laboratory. There is a clear difference between established high throughput routine haematology assays such as white blood cell count and
Haemoglobin and indirect measures such as the determination of Factor VIII. The results here also show the need to monitor measurement uncertainty regularly, with lot changes of calibrants, reagents and particularly internal QC. Combination of errors and propagation in combined uncertainty across multiple analysers is also required to be assessed by appropriate methods.

817 INTRODUCING IMMATURE GRANULOCYTES (IG) IN A PROFICIENCY TESTING PROGRAM AS A NEW PARAMETER ON AUTOMATED HEMATOLOGY ANALYZERS

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Introduction: Hematology analyzers have several new parameters for improved laboratory workflow and optimized reporting of patient results. One of these parameters is immature granulocytes (IG) which includes metamyelocytes, myelocytes and promyelocytes. Elevation of the IG may indicate sepsis, inflammation, trauma, cancer, metabolic abnormalities or myeloproliferative neoplasm. The Quality Management Program for Laboratory Services (QMP-LS) provides an external quality assessment program for laboratories. In 2012, a patterns-of-practice survey was conducted to determine current practice for reporting IG in order to include this parameter in the proficiency testing program.

Methods: An online survey was distributed to 182 participants with questions addressing the implementation of reporting IG’s including validation, determination of accuracy/bias and precision and training for interpretation prior to reporting patient results. Subsequently, a pilot assessment of the IG count separately from the granulocyte count was performed in a routine Hematology proficiency testing survey. Results: Of 182 participants, 31 (17%) reported that they had determined the clinical validity and verified accuracy/bias, 21 (11.5%) reported that they determined precision of the IG parameter on the hematology analyzers. Only 17 (9%) noted they currently include immature granulocytes in the patients’ reports. Of the 17, 2 were the Abbott Cell-Dyn 4000/ Sapphire analyzer, and 5 were the Beckman Coulter LH 500, which only provide flagging of IG’s and 10 were Sysmex instruments, including XE-2100/2100iC/5000i/XN and XT-2000i analyzers which enumerate IG. For the pilot survey, of 198 participating laboratories, 38 Sysmex users had capability performing the IG count and all 38 provided the IG value for this assessment. The results of the IG counts were all within allowable performance limits (±0.2 x10^9/L) (Table 1). Table 1. The results of absolute IG counts

<table>
<thead>
<tr>
<th></th>
<th>Total Number</th>
<th>Mean Result x10^9/L</th>
<th>SD</th>
<th>Percent CV</th>
<th>Minimum-Maximum Results x10^9/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>VIAL A</td>
<td>38</td>
<td>0.6</td>
<td>0.05</td>
<td>8.3</td>
<td>0.5-0.7</td>
</tr>
<tr>
<td>VIAL B</td>
<td>38</td>
<td>1.0</td>
<td>0.07</td>
<td>7.0</td>
<td>0.9-1.1</td>
</tr>
<tr>
<td>VIAL C</td>
<td>38</td>
<td>0.6</td>
<td>0.06</td>
<td>10.0</td>
<td>0.5-0.7</td>
</tr>
</tbody>
</table>

Conclusions: The reporting of new parameters poses challenges to proficiency testing since some laboratories are reporting and others are not. It is the responsibility of individual laboratories to ensure appropriate validations for any new parameter reported from automated hematology analyzers (such as the IG count). Laboratories should not only understand the clinical utility of these new parameters, but also potential sources of error and limiting factors prior to reporting. In addition, laboratories should provide proper notification and education of the clinical utility for these new parameters to the health care providers ensuring appropriate treatment is provided to patients based on the correct interpretation of laboratory results.

819 DEVELOPMENT AND VALIDATION OF ACCURATE AND EFFECTIVE METHOD BASED ON 99th PERCENTILE COEFFICIENT OF VARIATION CUTOFF VALUES OF DAILY MEANS FOR THE INTER-INSTRUMENT COMPARISON APPLICABLE TO AUTOMATIC HEMATOLOGY ANALYZERS

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Introduction: We developed the new inter-instrument comparison method based on 99th percentile coefficient of variation (CV) cutoff values of daily means, and validated the clinical relevance of this method in both patient samples and quality control (QC) materials. Methods: A total of 119-121 patient samples obtained for six months were enrolled. Samples obtained for the former three months were used in the determination of 99th percentile CV cutoff values in each item, and the data from the later three months were used for the calculation of daily acceptable ranges and rejection rates. Identical analysis was also performed with QC materials obtained for four months, and the acceptable ranges calculated from currently used method and 99th percentile CV cutoff values were compared. Two instrument comparison using patient samples were also performed, and the most appropriate allowable total error (ATE) values were determined. Results: The rejection rates in each item based on the 95%99th percentile cutoff values were 1.67% - 21.67%/0.00% - 10.00% in patient samples and 0.00% – 16.28%/0.00% - 9.30% in QC materials, respectively, and the rejection rates were significantly reduced when the 99th percentile CV cutoff values were applied instead of 95th percentile CV cutoff values. The acceptable ranges of QC materials based on currently used method included those calculated from the 99th percentile CV cutoff values in most of items. In two-instrument comparisons, 34.8% of all comparisons such as differential counts, reticulocytes and mean corpuscular volume (MCV) were failed, and 87.0% of failed comparisons passed when 4SD values are applied as an ATE instead of currently used 3SD. Conclusions: The 99th percentile CV cutoff values-derived daily acceptable ranges can be used as a real-time inter-instrument comparison method applicable to both patient samples and QC materials. When the QC materials...
are used, the 99th percentile CV cutoff values should be used for the calculation of acceptable ranges rather than in-house derived ranges. In the differential counts, reticulocytes and MCV, the application of 4SD as an ATE value in two-instrument comparison can significantly reduce the frequency of unnecessary recalibration.

821 PRELIMINARY STUDY TO SEE THE EFFECT OF IN VITRO HEMOLYSIS ON COMPLETE BLOOD COUNT PARAMETERS

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Introduction: Hemolysis is one of the most encountered preanalytical errors in clinical laboratories. It is one of the quality indicators of the preanalytical phase. Some labs choose to reject the affected parameters while some choose to reject the whole sample. However, only a few labs reject the whole blood sample drawn for complete blood count (CBC) due to hemolysis. This study was planned to investigate the effect of in vitro hemolysis on each CBC parameter.

Methods: We included EDTA anticoagulated whole blood samples of 25 patients for this study. The samples were first studied on the Mindray 6800 system for CBC. Then, they were sunk in a water bath which was adjusted to 60°C and kept for 1 minute. Then, they were taken out, and after reaching the room temperature, ran on the same analyzer for CBC for the second time. Student’s t-test and Wilcoxon signed rank test were used for the comparison of the means of the two groups. Spearman (r) and Pearson correlation analyses were used to determine the mathematical correlations between variables. Significance level was set at p<0.05.

Results: After the in vitro hemolysis process, all of the parameters were significantly affected (p<0.05) except white blood cells, neutrophils, and lymphocytes (p>0.05). Also, the correlation analyses revealed statistically significant correlation before and after the hemolysis in the hemoglobin, hematocrit, platelet, MCV, MCH, RDW, eosinophil, PCT, and PDW parameters (p<0.05).

Conclusions: Hemolysis should be encountered as an important preanalytical error in hematology laboratories. It is not a negligible indicator of preanalytical error in CBC labs.

6) Platelet count correlation: XE impedance v reference, R² = 0.984, intercept +4.42  XE Optical v reference, R² = 0.992, intercept +11.34  XN PLT-F v reference, R² = 0.997, intercept +0.39  n= 11/16 plt < 50 x 10⁹/L (XE impedance) Blood film: RBC fragmentation/ anisocytosis/ dimorphism/ WBC pyknosis/ large platelets on 12/16.

Conclusions: LW mode: accurate WBC count and differential. WPC ‘blasts’ flag - greater sensitivity than XE5000, specificity and overall efficiency similar. WPC increased specificity and overall flagging efficiency over WDF channel alone, blood film review reduced. WPC reflex testing removed WDF ‘Blasts/ Abn lympho’ flag on 46% samples. Good correlation (R²) for platelet methods against reference, however XE optical channel had much higher intercept value than XN PLT-F on samples with known interferents, potentially affecting clinical decisions regarding platelet transfusion. Work ongoing.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Prehemolysis (mean±SD)</th>
<th>Posthemolysis (mean±SD)</th>
<th>p value</th>
<th>Correlation coefficients and p values of pre- and posthemolysis</th>
<th>Correlation coefficient</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin</td>
<td>13.8±6.82</td>
<td>15.2±7.1±1.01</td>
<td>&lt;0.0001</td>
<td>0.521</td>
<td>0.008</td>
<td></td>
</tr>
<tr>
<td>Hematocrit</td>
<td>41.8±2.43</td>
<td>44.9±2.28</td>
<td>&lt;0.0001</td>
<td>0.516</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>Platelet</td>
<td>289.9±105.56</td>
<td>225.6±16.4</td>
<td>0.013</td>
<td>0.514</td>
<td>0.009</td>
<td></td>
</tr>
<tr>
<td>MCV</td>
<td>80.8±9.3±10</td>
<td>50.0±94.23</td>
<td>&lt;0.0001</td>
<td>0.517</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>RDW</td>
<td>13.5 (12.5-15.8)</td>
<td>13.5 (12.8-16.2)</td>
<td>0.332</td>
<td>0.532</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
</tbody>
</table>

*Due to the nonhomogeneous distribution of RDW values, median(min-max) values are given and Wilcoxon signed rank test and Spearman correlation analysis were performed.
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