Abstracts

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European Union of Medical Specialists, Section of Laboratory Medicine/Medical Biopathology
Polish Society of Laboratory Diagnostics

Under the auspices of
International Federation of Clinical Chemistry and Laboratory Medicine
International Association of Therapeutic Drug Monitoring and Clinical Toxicology,
Collegium Medicum of Nicolaus Copernicus University, Warsaw Medical University

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   Laboratory in Emergency Department  
   Laboratory testing in pregnancy  
   Metabolic diseases  
   Microbiology  
   New technologies  
   Protein assays  
   Thyroid disorders
Clinical usefulness of bone turnover marker levels in osteoporosis: 2016 update

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Chair, IFCC-IOF WG for Standardisation of Bone Marker Assays

A wide range of biochemical markers provide information on the activities of bone cells, largely osteoclasts and osteoblasts, commonly termed bone turnover markers (BTM). They divide into two main categories, markers of bone cells and type I collagen formation or breakdown products. Robust assays for many markers have been adapted to automated biochemical platforms making them rapidly and cost-effectively available in clinical laboratories. Recent evidence confirms their usefulness for monitoring response to antiresorptive therapies for osteoporosis. Their role for providing prognostic information on risk of fracture is uncertain however. The specificity of BTMs for bone cell activities has markedly increased over recent times. For example the assay for C-telopeptide fragments of collagen type I α1 chains (serum Crosslaps (ß-CTX)) is specific for not only the cross-linking of type 1 collagen between lysine residues in adjacent type I collagen molecules but also for the isomerization of the arginyl peptide to the β-form which can only take place in the mature collagen of bone. This marker therefore is highly specific for bone resorption. A major limitation for their clinical usefulness is their marked variability. Pre-analytical variation arises from the use of serum versus plasma samples or the urine collection procedure. Analytical variability can arise from assays from different manufacturers. There is a large biological variability depending on the status of the subject including seasonality, sex, age and menopausal or disease status. BTM levels can vary across the 24-hour period particularly associated with food intake. These acute factors are more significant for bone resorption BTMs and probably reflect acute changes to osteoclast activities although such changes have not been clearly proven. Disclosure: Funds and reagents have been provided to the IFCC-IOF WG-SBMA by Roche Diagnostics, Immunodiagnostic Systems Ltd and Orion Diagnostica.

ECTS lecture on bone biomarkers

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‘Biomarkers of bone metabolism’ are substances derived from almost all processes of bone activity. Systemic dispersion is the basis for their biochemical or molecular measurements. Both serum/plasma and urine measurements have been widely used as clinical parameters of bone turnover, but accurateness and usability have favoured blood analyses over the years. This lecture will therefore focus on serum/plasma parameters of bone metabolism and their clinical applications including future aspects using appropriate biological material. Current targets of the analysis of bone metabolism are enzymes and proteins or their respective metabolites produced during bone formation and degradation in health and disease. However, direct regulators of osteoblast and/or osteoclast cell function and activity might represent additional parameters of interest, since they may very well reflect dynamic processes in bone and adjacent tissue. The usefulness of bone biomarkers has been widely recognized during the past decades, but increased only recently with the technical improvement of automated standardized methods and the knowledge of new compounds in bone metabolism. Many of these markers have been tested for practical use, and some of them have already entered daily clinical routine applications. The reason for this increasing interest is the characterization and diagnosis of metabolic bone diseases, but also the improvement of therapy selection and monitoring by biomarkers as documented in recent studies.

This lecture aims to describe all types of bone biomarkers and the pros and cons of their clinical use in bone diseases including standardization and interpretation issues - future aspects and new approaches will be discussed.

Clinical use of bone turnover markers in Central Europe

Roman Lorenc (PL)

No abstract available
Practical aspects of measurements of bone turnover markers, sources of variability, standardization of methods

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The field of bone turnover markers has developed considerably in the past decade. Various biochemical markers are now available that allow a specific and sensitive assessment of the rate of bone formation and bone resorption of the skeleton. Although these markers are not recommended for use in diagnosis of osteoporosis yet, they appear to be useful for the individual monitoring of osteoporotic patients treated with antiresorptive agents. The relationship between biochemical markers of the bone turnover and the rate of bone loss may show conflicting results because of the various limitations. These limitations include considerable short- and long-term biological fluctuations (e.g., diurnal variability, age and gender), lifestyle and diet, as well as technical variability (inter- and intra-laboratory sample collection and analysis). These shortcomings need to be addressed to optimize the use of bone turnover markers in clinical practice.

Opening plenary

Plasma DNA: Driver of a revolution in molecular diagnostics for the clinic

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Human plasma contains a mixture of nucleic acids released from multiple organs and tissues within the body. The analysis of these nucleic acid species has created a revolution in molecular diagnostics. One such type of circulating nucleic acids is DNA that is released by a fetus into the plasma of its mother. The analysis of such circulating fetal DNA has made possible the current global practice of non-invasive prenatal testing (NIPT). Another type of circulating nucleic acids is DNA released by tumour cells into the plasma of a cancer patient. Such circulating tumour DNA has opened up one way by which one can perform the ‘liquid biopsy’ of tumours. Such liquid biopsies allow clinicians to detect, to choose the optimal treatments for, to monitor, as well as even to screen for cancers. Recent advances in genomics and DNA sequencing technologies have allowed one to analyse such circulating nucleic acids with a breadth and a depth that would not be practical before. This lecture will provide an overview of the exciting developments in this area, as well as looking forward to future developments in the coming years.

Thursday, September 22nd, 2016

Parallel sessions

Session 1: Laboratory biomarkers of cardiovascular disease

High sensitive cardiac troponin assays: from improved analytical performance to enhanced risk stratification

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Implementation of cardiac troponin (cTn) assays has revolutionized the diagnosis, risk stratification, triage and management of patients with suspected myocardial infarction (MI). Currently, detection of a rise and/or fall in concentration of myocardial necrosis biomarkers, preferentially cTns, with at least one value above the 99th percentile upper reference limit, is the essential component for the diagnosis of MI. High-sensitivity cardiac troponin (hs-cTn) assays with their superior analytical performance were designed to further facilitate clinical decision making. This presentation will aim to integrate updated laboratory and clinical knowledge regarding hs-cTn assays in order to promote their optimal use in daily practice. It will primarily focus on i) the role of hs-cTn assays in patients with suspected MI, discussing the recommended diagnostic algorithms and result interpretation, and on ii) the prognostic value of hs-cTn tests, including non-coronary causes of cTn elevation. Emphasis will be also placed on the release of cTn following myocardial injury, analytical performance of hs-cTn assays, and potential challenges related to the selection of healthy reference population in determining 99th percentile values. This presentation underscores the need for education of clinicians and medical laboratory professionals regarding appropriate use and interpretation of hs-cTn assays. Adequate training and clinical experience with these tests are essential for translation of improved analytical performance of hs-cTn assays into enhanced risk stratification and hopefully better patient outcomes.
Biomarkers in heart failure: Natriuretic peptides and beyond

Rudolf de Boer
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Biomarkers are intensively investigated in the field of heart failure. Natriuretic peptides are successfully integrated into the clinical practice of heart failure for diagnosis, and in the future possibly to guide treatment. Now, the possibility of using new biomarkers to advance the management of affected patients is actively explored. While a huge number of candidate heart failure biomarkers have been recently described, very few have made the difficult translation from initial promise to clinical application. But we have several of such markers, and they mirror the complex pathophysiology of heart failure at various levels: cell loss (troponin), fibrosis (ST2 and galectin-3), infection (procalcitonin), and renal disease (several renal markers). In this presentation, we will discuss the best established and emerging candidates for clinical assessment and management of patients with heart failure.

Role of biomarkers in the diagnosis and risk stratification of patients with suspected pulmonary embolism

Maciej Kostrubiec
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Venous thromboembolism (VTE) includes deep vein thrombosis (DVT) and pulmonary embolism (PE). It is the third most frequent cardiovascular disease with an overall annual incidence of 100–200 per 100 000 inhabitants. Acute PE is the most serious clinical presentation of VTE, it can be lethal in the acute phase or lead to chronic disease and disability, but it is also often preventable. Biomarkers play an important role in diagnosis and risk stratification of stable patients with suspected pulmonary embolism without hypotension or shock.

PE may diagnosis may be difficult since the clinical signs and symptoms are non-specific. When the clinical presentation raises the suspicion of PE in an individual patient, it should prompt further objective testing. In most patients, PE is suspected on the basis of dyspnoea, chest pain, pre-syncpe or syncpe, and/or haemoptysis. Despite the limited sensitivity and specificity of individual symptoms, signs, and common tests, the combination of findings evaluated by clinical judgement or by the use of prediction rules allows to classify patients with suspected PE into distinct categories of clinical probability that correspond to an increasing actual prevalence of confirmed PE.

D-dimer levels are elevated in plasma in the presence of acute thrombosis, because of simultaneous activation of coagulation and fibrinolysis. The negative predictive value of D-dimer testing is high and a normal D-dimer level renders acute PE or DVT unlikely. On the other hand, fibrin is also produced in a wide variety of conditions such as cancer, inflammation, bleeding, trauma, surgery and necrosis. Plasma D-dimer measurement is recommended in outpatients and emergency department patients with low or intermediate clinical probability to reduce the need for unnecessary imaging and irradiation, preferably using a highly sensitive assay. In low and moderate clinical probability normal D-dimer level using a highly sensitive assay excludes PE. However, D-dimer measurement is not recommended in patients with high clinical probability, as a normal result does not safely exclude PE, even when using a highly sensitive assay.

Right ventricular pressure overload in acute PE is associated with increased myocardial stretch, which leads to the release of brain natriuretic peptide (BNP) or N-terminal (NT)-proBNP. The plasma levels of natriuretic peptides reflect the severity of haemodynamic compromise and RV dysfunction in acute PE. On the other hand, low levels of BNP or NT-proBNP can identify patients with a favourable short-term clinical outcome based on their high negative predictive value.

Similarly, elevated plasma troponin concentrations on admission have been reported to be associated with worse prognosis in acute PE. The reported positive predictive value of troponin elevation for PE-related early mortality ranges from 12–44%, while the negative predictive value is high, irrespective of the assays and cut-off values used. Recently developed high-sensitivity assays have even improved the prognostic performance of this biomarker, particularly with regard to the exclusion of patients with an adverse short-term outcome.

Patients who display evidence of both RV dysfunction (by echocardiography or CT angiography) and elevated cardiac biomarker levels in the circulation (particularly a positive cardiac troponin test) should be classified into an intermediate-high-risk category. While patients in whom the RV is normal on echocardiography or CT angiography, and/or have normal cardiac biomarker levels, belong to an intermediate-low-risk group. Haemodynamically stable patients without significant comorbidities, with low BNP or NT-proBNP levels, negative troponin test and no signs of RV dysfunction on an imaging test have the low risk of early death and may be candidates for early discharge and outpatient treatment.

Short communications

Additive value of clopidogrel-pathway gene polymorphisms to clinical risk-stratification of patients with ST-segment elevation myocardial infarction undergoing primary PCI

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Objective: The present study aims at examining the association between clopidogrel-pathway gene polymorphisms, on treatment platelet reactivity and the RISK-PCI score on risk prediction of 30 day major adverse coronary event (MACE) in patients with ST-elevation acute myocardial infarction (STEMI) treated by primary percutaneous coronary intervention (PCI).

Methods: Random sample of 140 patients consecutive patients referred to primary PCI for STEMI in a high-volume cath lab were recruited. The platelet function was assessed by Multiple Electrode Aggregometry. The clopidogrel-metabolizing pathway SNPs used were: ABCB1 (rs1045642), CYP2C19*2 (rs4244285), CYP2C19*17 (rs12248560), P2RY12 (rs2046934), and PON1 (rs854560, rs662). The primary end point MACE was defined as death, nonfatal infarction or immediate target vessel revascularization. Patients were followed-up at 30 days after primary PCI.

Results: Thirty-day MACE was 4.3%. All SNPs tested were in Hardy-Weinberg equilibrium (p > 0.05). Among the SNPs tested, only CYP2C19*17 was significantly associated with MACE, despite no significant association with platelet activity. Addition of CYP2C19*17 T allele to RISK-PCI score increased the area under the ROC (0.581 vs. 0.857). The addition of CYP2C19*17 T allele to RISK-PCI score enhanced net reclassification improvement (1.357, P < 0.001) and integrated discrimination improvement (0.210, P < 0.05), suggesting effective discrimination and reclassification.

Conclusion: These data revealed the combination of the established RISK-PCI score and CYP2C19*17 could derive a more accurate prediction for clinical outcomes in STEMI patients.

Soluble urokinase plasminogen activator receptor in patients after first myocardial infarction – prediction or diagnostic marker?

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Introduction: Soluble urokinase plasminogen activator receptor (suPAR) level reflects general condition of the organism and was proved to give independent information in risk stratification of patients. The aim of this study was to assess the usefulness of suPAR in the prediction of major adverse cardiac events (MACE) in patients with first myocardial infarction (MI) undergoing primary percutaneous coronary intervention. Additionally the diagnostic power of suPAR was assessed.

Material and Methods: 139 of 150 consecutive patients were included. Serum suPAR (ELISA, Virogates) as well as CRP (on admission and at discharge) and maximum cardiac troponin T (from each following 6-hour period of blood collection) were measured. Left ventricular ejection fraction (LVEF) and wall motion score index (WMSI) were assessed in echocardiography. In the one-year follow up study the following MACEs were observed: non-fatal MI, revascularisation, stroke and death.

Results: Multi-variable analysis revealed prognostic usefulness only for suPAR and glomerular filtration rate: p: < 0.0001 and 0.018 respectively; OR: 2.59 and 0.98 respectively, with AUC in ROC analysis for both parameters simultaneously 0.89, p < 0.0001. There was no correlation between suPAR and LVEF, WMSI, nor maximum cTnT or MI type. There was a statistically relevant difference in suPAR concentrations after 1 year (4.15 ng/mL and 5.37 ng/mL, p < 0.0001).

Conclusion: suPAR appears to be an independent biomarker for the prediction of MACE, early after first myocardial infarction. Lack of correlation between suPAR and left ventricular systolic function parameters as well as parameters associated with MI may confirm suPAR has more prognostic than diagnostic power.

Variability of Heart Failure Biomarkers; Principles for proper interpretation

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Aims: Biomarkers may be used for diagnosis, risk stratification or management of patients with heart failure (HF). Knowledge about the biological variation is needed for proper interpretation of serial measurements. Therefore, we aimed to determine and compare the biological variation of a large panel of biomarkers in healthy subjects and in patients with chronic HF.

Methods and Results: The biological variability of established (NT-proBNP, hsTroponinT), novel (Galectin-3, ST2, GDF-15) and renal/neurohormonal (Aldosterone, Phosphate, Parathyroid hormone, Plasma Renin Concentration, Creatinine) biomarkers was determined in 28 healthy subjects and 83 HF patients, over a period of 4 months and 6 weeks, respectively. The analytical (CVa), intra-individual (CVi), and inter-individual (CVg) variations were calculated, as well as the reference change value (RCV), which reflects the percentage of change that may indicate a “relevant” change.

All crude biomarker levels were significantly increased or decreased in HF patients, compared to controls (all P < 0.01). Variation indices were comparable in healthy individuals and in HF. CVi was not influenced by the individual levels of the biomarker itself. NT-proBNP and
GDF-15 had relatively high CVi (21.8% and 16.6%) and RCV (61.7% and 64.3%), whereas ST2 (CVi: 15.1; RCV: 42.9%), hsTnT (CVi: 11.1; RCV: 31.4%), and galectin-3 (CVi: 8.1; RCV: 25.0%) had lower indices of variation.

Conclusion: Biological variation indices are comparable between healthy subjects and HF patients for a broad spectrum of biomarkers. NT-proBNP and GDF-15 have substantial variation, with lower variation for ST2, hsTnT, and galectin-3. These data are instrumental in proper interpretation of biomarkers levels in HF patients.

Session 2: Diabetic kidney disease: beyond albuminuria

Monogenic diabetes: Screening and diagnosis

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No abstract available

Will standardisation of urine albumin provide us with a more reliable test for the detection of early diabetic kidney disease?

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Urine albumin is one of the accepted markers of diabetic kidney disease. There is evidence to support the association between increasing urine albumin and the progression of chronic kidney disease (CKD) to end stage renal disease (ESRD). There is some evidence to suggest that the treatment of urine albumin with ACE-I may reduce cardiovascular outcomes in patients with diabetes. The worldwide incidence of diabetes continues to increase and it is the leading cause of ESRD in Western Countries, requiring treatment through dialysis or kidney transplant. With up to 50% of patients with diabetes developing diabetic kidney disease, the use of a biomarker to identify CKD early is important. Urine albumin values >2.0 mg/mmol creatinine (~30 mg/day) have prognostic significance for the development of CKD and have been included in the Kidney Disease Improving Global Outcomes (KDIGO) and other clinical practice guidelines as part of the diagnostic criteria for CKD. There are analytical considerations related to urine albumin measurement that need to be taken into consideration by the clinical laboratory. The evidence supporting the use of urine albumin is not based on assays in routine clinical laboratory use. The IFCC has been working on standardizing the measurement of urine albumin and this is an ongoing project. Currently available methods for urine albumin and urine creatinine can lead to diagnostic differences and potentially result in delayed management of patients. Standardisation of methodology, terminology and reporting of urine albumin may make this an even better marker than what it is at the moment.

Diabetic Kidney Disease: Beyond Albuminuria

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In the early 1980s several groundbreaking epidemiologic studies have described the natural history of diabetic nephropathy from its earliest clinically traceable manifestations. As a result, mildly elevated albumin level in urine (microalbuminuria), for the next several decades, became the sole clinical marker of early kidney injury, indicating elevated risk of progression towards more advanced stages of diabetic kidney disease. It also became a therapeutic target of renoprotective interventions. Recent epidemiologic studies and results of clinical trials indicate that albuminuria is currently less strongly coupled with disease progression than it was previously perceived. Current renoprotective treatments strongly reduce albuminuria, but are less effective in preventing loss of renal function and end-stage renal disease, therefore, albuminuria cannot be used anymore as the primary end-point in clinical trials. New disease markers are urgently needed for early disease detection, risk stratification, progression monitoring and evaluation of response to treatment. New markers are necessary to serve as surrogate end-points in clinical trial evaluating new therapies of diabetic kidney disease, and they must remain similarly strongly correlated with clinical end-point (end-stage renal disease) in both treatment and placebo arms. Dozens of particles, small molecules, peptides, proteins and micro-RNAs show strong association in prospective studies with adverse renal outcomes. Recent advances in high-throughput proteomic and metabolomics assays have greatly sped up biomarker detection process. Several particles seem especially promising: soluble tumor necrosis factor receptors in serum, serum kidney injury molecule, urinary macrophage chemoattractant protein-1 in urine or soluble urokinase receptor in plasma. Although many markers are clinically redundant, several of them in combination, together with urinary albumin/creatinine ratio provide improved prediction of diabetic kidney disease progression. They can also provide some insight into disease mechanisms, such as renal injury, inflammation, and fibrosis.
as glomerular and tubular damage, matrix expansion or inflammation. Approval of biomarker-based surrogate end-points for clinical trials is not expected soon. Currently the focus has shifted from albuminuria to surrogate end-points based on serial measurements of glomerular filtration rate, where the slope of renal decline seem to be the most advantageous. Diligent monitoring of renal function is currently the best option for early risk detection in clinical practice.

**Diabetes and sTumour necrosis factor receptor “in the Wild”**

*Pat Twomey (IRL)*

No abstract available

**Short Communication**

**Urine electrophoresis suggests different mechanism of early renal impairment among patients with type 2 diabetes**

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Background and aim: Diabetic kidney disease (DKD) was thought to be primary glomerular. However, findings regarding type 2 diabetes (T2DM) suggest other mechanisms of renal impairment in some patients, including primary tubular dysfunction. The aim of the study was to characterize the patterns of proteinuria in T2DM patients at risk of and with early DKD.

Methods: We recruited 80 patients with T2DM (38 men, 42 women; mean age 61 ± 12 years), with eGFR ≥60 ml/min/1.73m² and urine albumin to creatinine ratio (uACR) ≤300 mg/g. First morning urine samples were collected for SDS-electrophoresis on polyacrylamid gels. Serum and urine uromodulin, urine transferrin and immunoglobulin G (IgG) were measured by immunochemistry.

Results: Proteinuria was detected by SDS-electrophoresis in 24 (30%) patients. Three different patterns of proteinuria were observed: selective glomerular in 15 patients, tubular in 3, and detectable uromodulin in 6. In 56 patients, there were only trace concentrations of albumin or no detectable protein in urine. Patients with selective glomerular proteinuria had significantly higher glucose and glycated hemoglobin, C-reactive protein, uACR, urine transferrin and IgG. Those with tubular proteinuria were older, had higher urine transferrin, and lower serum uromodulin. Uromodulin was detected by electrophoresis in younger patients; this pattern was associated with higher concentrations of uromodulin both in urine and in serum.

Conclusions: SDS-electrophoresis of urine allows for the diagnosis of different pathomechanisms associated with early DKD. Selective glomerular and tubular proteinuria are observed in patients with different clinical characteristics.

**Session 3:Therapeutic drug monitoring and pharmacogenetics of immunosuppressants**

**Modern analytical methods for routine determination of immunosuppressive drugs**

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Therapeutic drug monitoring (TDM) plays a crucial role in individualized immunosuppressive therapy. The treatment is based on simultaneous administration of drugs with complementary mode of action and therapeutic schemes is dependent on transplant organ and patient’s characteristics. Among primary immunosuppressive drugs (ISD), cyclosporine, tacrolimus, everolimus and sirolimus require whole blood monitoring, whereas mycophenolic acid (MPA) needs to be determined in plasma. Currently, methods for measuring ISD are divided into chromatographic ones and into automated immunoassays. It is generally well recognized that a properly validated chromatographic method is superior to immunoassay because of its specificity. However, the choice of analytical technique is very dependent on resources available and on the number of samples to be analyzed by the laboratory. The reference chromatographic techniques are preferred at larger TDM centers employing highly educated and trained analysts, often located at academic hospitals. Small, local or transplant oriented medical laboratories prefer rather ready-to-use, automated immunoassays easily offered by analytical companies. So far, medical diagnostic laboratories in many countries did not benefit enough from new chromatographic tools, especially liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). These modern analytical methods, generally not used for laboratory diagnostics in Poland mainly due to expensive apparatus.
and common opinion that they are too sophisticated for routine analyses, are recently becoming more attractive because of shortening turnaround time, lowering reagents consumption and decreasing equipment price.

Pharmacokinetic tools for the dose optimization during immunosuppressive therapy

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Anti-rejection therapy in organ transplantation has long been a privileged application field for the development of medicine personalization, owing to the large variability in transplant patient and graft outcomes, the narrow therapeutic margin of most immunosuppressive drugs (ISD), their unavoidable pharmacokinetic (PK) interactions and the unescapable long term toxicity of most ISD.

The variability in the therapeutic and toxic effects of ISD obviously has PK and pharmacodynamic (PD) origins, both with genetic and environmental causes. Over the last two decades, patient care has been improved by using PK monitoring in order to identify the sources, and compensate as much as possible for, the variability of ISD therapeutic and adverse effects. This has involved population pharmacokinetic (popPK) analysis and modelling, Bayesian estimators of exposure and dose adjustment tools for calcineurin inhibitors, mycophenolates and to a lesser extent, mTOR inhibitors (some of which have been validated clinically and made accessible through websites). There is currently renewed interest in PK tools of dose adjustment owing to the recent approval of prolonged release formulations of tacrolimus, to investigate new potential sources of PK variability, in particular regarding the absorption phase, as well as to help clinicians to better handle these new formulations.

Among the many polymorphisms in genes coding for ISD metabolizing enzymes or efflux transporters explored, only a few have shown a strong enough impact on PK to be taken into account for a priori (before the first dose) or a posteriori (based on measured concentrations) dose adjustment, and/or have been proposed as covariates in popPK models.

Finally, pharmacokinetic tools have proved to be clinically useful and represent a first and important step in treatment personalization of ISD.

The role of pharmacogenetics in therapeutic drug monitoring of immunosuppressive drugs

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The clinical use of the immunosuppressant tacrolimus is complicated by considerable toxicity, a narrow therapeutic window and marked variability in its pharmacokinetics. Therapeutic drug monitoring (TDM) is now routinely used to optimize tacrolimus therapy in most transplant centers throughout the world.

Tacrolimus is metabolized by cytochrome P450 (CYP) 3A4 and CYP3A5. Variation in the expression and/or activity of these metabolizing enzymes has been recognized for a long time as an important cause of the high inter-patient variability in tacrolimus exposure. In the last decade, several single-nucleotide polymorphisms (SNPs) were discovered in the CYP3A4 and CYP3A5 genes. These SNPs were subsequently shown to explain part of the variability in tacrolimus dose requirement between individual patients.

Now that a more sophisticated prediction of an individual’s tacrolimus metabolizing phenotype has become possible, the question arises whether pharmacogenetics should be incorporated in clinical practice as an adjunct to classic TDM. Recently, two pharmacogenetic randomized-controlled clinical trials were conducted with the aim to investigate whether a CYP3A5 genotype-based approach to tacrolimus dosing has the potential to improve clinical outcomes of kidney transplantation. These studies have yielded conflicting results, with one trial demonstrating a benefit of CYP3A5-based tacrolimus dosing, whereas the other did not. It is envisaged that in the future, tacrolimus dosing will be guided by algorithms which incorporate demographic, clinical and genetic information on multiple genes involved in the pharmacokinetics of tacrolimus. However, evidence from clinical studies is needed before the implementation of such a strategy can be fully justified.

Short Communications

Effect of hypoalbuminemia on plasma protein binding of mycophenolic acid in lupus nephritis patients

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Background: Mycophenolic acid (MPA) is strongly bound to albumin (97-99%), thus only 1-3% of free MPA is pharmacologically active in patients with normal liver and kidney function. Certain conditions may cause free MPA increase with minor effect on total MPA concentrations [1, 2]. Therefore, the aim of this study was to assess the effect of hypoalbuminemia on MPA plasma protein binding in lupus nephritis patients.

Methods: Twenty two abbreviated profiles (C0, C0.5, C2) were provided from twelve lupus nephritis patients on mycophenolate mofetil therapy. Concentrations of total MPA in plasma and free MPA in plasma ultrafiltrate were measured using HPLC-UV and LC-MS/MS methods, respectively. Free fraction of MPA was calculated by dividing C free by C total (x 100%).

Results: Median MPA free fraction calculated for all sample time points was 0.94% (range: 0.05-6.37%), while the mean (±SD) plasma albumin concentration was 4.07 ± 0.72 g/dL. The significant correlation between plasma albumin concentration and free fraction of MPA was observed (C0: r = -0.4228, p = 0.0499; C0.5: r = -0.5056, p = 0.0164; C2: r = -0.4346, p = 0.0490), what indicated that when albumin concentration decreased the elevated MPA free fraction was observed.

Conclusions: Monitoring of free MPA concentrations should be considered in lupus nephritis patients with hypoalbuminemia, as in these patients it may estimate exposure on MPA better than total MPA monitoring. These findings should be further confirmed in larger clinical studies.

Triangulation of data between phenotype thiopurine methyltranferase activity, TDM of azathioprine metabolites and genotyping for variant alleles in patients undergoing azathioprine therapy

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Aim: A I was conducted of episodes of requests for thiopurine methyltransferase (TPMT) activity in patients proposed for azathioprine (AZA) therapy. The aim was to evaluate the effectiveness of current ‘cut-offs’ that trigger reflexive genotyping requests on patients that appear to demonstrate impaired enzymatic activity. The laboratory currently sends-away referrals for metabolite concentrations TDM but a further aim of the study was to determine whether a local TDM service providing timely relative thioguanine nucleotide (TGN) and methylmercaptopurine nucleotide (MMPN) concentrations could contribute to decision-making related to reflexive genotyping, in addition to improving patient care.

Methods: Data extraction was performed on the Laboratory Information System (LIS) for patient episodes of TPMT activity requests. Episodes were filtered to exclusively contain patients who also had data related to TDM metabolite concentrations after the initiation of AZA therapy, leading to 175 cases. Finally, episodes of reflexive genotyping among the filtered data were reviewed.

Results: Low TPMT activity leading to higher TGN concentrations compared with MMPN was presented as the hypothesis. However, there was poor correlation between measured TPMT activity and the relative ratio of TGN and MMPN concentrations. Some cases of low TPMT activity did not demonstrate variant allelles, and there were cases of high relative levels of TGN compared with MMPN where TPMT activity appeared to be normal and which did not trigger genotyping.

Conclusion: A local TDM service for measuring AZA metabolites could contribute to genotyping decision-making for variant allelles determining TPMT activity.

WORKSHOPS

WS1: ALEXION

Recognition of low alkaline phosphatase activity: The role of the laboratory in the clinical diagnosis of hypophosphatasia

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WS2: The diagnostic and prognostic role of high sensitivity cardiac troponin I.

Abbott Diagnostics

Rapid rule-out of acute myocardial infarction and gender-specific diagnosis

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This presentation will review the impact of contemporary sensitive and high-sensitivity troponin assays on management and clinical outcomes in patients with suspected acute coronary syndrome. The Abbott ARCHITECT STAT high-sensitivity cardiac troponin I assay has excellent precision at troponin concentrations below the 99th percentile and will permit implementation of the universal definition of myocardial infarction with greater confidence. Issues that need to be considered during implementation of a high-sensitivity cardiac troponin assay into clinical practice will be covered. First, high-sensitivity troponin assays have identified differences in the reference range for troponin between men and women raising the possibility that we may have been under diagnosing myocardial infarction in women. Should we use sex-specific diagnostic thresholds in men and women with suspected acute coronary syndrome? Second, what is the optimal time to measure cardiac troponin and how should we use serial troponin measurements to improve diagnostic accuracy? Finally, how can we use a high-sensitivity cardiac troponin assays to reliably identify patients without myocardial infarction at presentation who may be suitable for discharge from the emergency department?

Short and long-term prognostic value of hsTnI

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The nearly absolute cardiac specificity of the immunoassays for cardiac troponin (cTn) has allowed to widespread their use as a valuable support for diagnosing myocardial infarction (MI). While the rough equivalency “positive cTn equals MI” is erroneous, though still difficult to be erased from many physicians’ attitude, a dynamic view of cTn testing has allowed a better diagnosis. The newest definition of myocardial infarction is centered on the rise and fall of cardiac troponin, and the recent ESC guidelines have provided a detailed guidance to serial testing for the diagnosis of MI without elevation of the ST segment (NSTEMI).

At the same time, troponin assays have evolved dramatically towards the status of high sensitivity (hs). This is defined as the capability of detecting cardiac troponin in at least 50% of a reference, healthy population while attaining a high precision (<10% total CV) at the 99th percentile of that population. There is currently only one commercial hs assay for troponin I (Abbott ARCHITECT hsTnI), and several papers have described its usefulness and accuracy in ruling out and ruling in MI. At the same time, the increased sensitivity has provided a useful insight on the prognostic value of hsTnI both in the acute setting and on long-term studies. Observational studies in real-life settings have linked hsTnI levels to death and MACE within 30 days from presentation in an acute setting, whereas cohort studies in open population have allowed assess its predictive value for long-term adverse outcomes at levels below the 99th percentile.

WS3: Greiner bio-one

Standardized blood sampling throughout Europe: Dream or reality

Implementing standardized venous blood sampling practices in one University hospital in Austria – pilot study

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As the EFLM working group Preanalytical Phase (EFLM WG-PRE) is currently drafting a guideline for a standardized venous blood collection and sample handling including recommendations for the respective implementation within a health care facility, a pilot study was needed to evaluate the practicability of this document. Since a similar project was planned on the pediatric wards of the University hospital of Salzburg (Austria), this site was chosen for such a study. Phlebotomy on these wards is mostly performed by physicians and plans are to shift this task to the nursing staff. Along with the currently developed guideline, a phlebotomy observational sheet, a PowerPoint presentation containing information about preanalytics and venous blood collection, and a respective knowledge test will be made available. Based on these documents an e-learning module is being implemented in the University hospital of Salzburg with the possibility of a single user login. All 240 pediatric nurses will be advised to go through this module including the final knowledge test with a mandatory positive result higher than 69%. All results will be documented and the module will have to be redone biennially. Additionally phlebotomy will be practiced using demo arms under the guidance and supervision of experienced nurses, who were all educated accordingly. These trainings will be offered 4-8 times per year, depending on the demand. Respective practical skills will be evaluated on a regular basis using the mentioned observational sheet. Sample quality is being monitored by the laboratory using preanalytical quality indicators as proposed by the IFCC (1-3).

References

WS4: Siemens Healthcare

Effective management of Graves disease

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Objectives:
- Understand the latest trends in how clinicians diagnose and monitor Graves’ disease
- Review the latest diagnostic tools recommended for proper differential diagnosis of hyperthyroidism and patient monitoring challenges
- Learn how the Thyroid Stimulating Immunoglobulin (TSI) assay can lead to faster, more accurate diagnosis and overall more efficient and less costly patient care

Background: Hyperthyroidism affects approximately 1.5% of the worldwide population. Graves’ disease is the most common cause of hyperthyroidism, and accounts for 60 to 80 percent of all cases. It is an autoimmune disorder caused by the thyroid stimulating antibody (TSI), active against the thyroid-stimulating hormone (TSH) receptor, which stimulates the gland to synthesize and secrete excess thyroid hormone. Being able to quickly and accurately diagnose the cause of hyperthyroidism is critical to the quality of patient care.

Methods: Fast and accurate differential diagnosis of hyperthyroidism is vital in order to initiate the appropriate treatment as soon as possible. Patient history, physical examination, and diagnostic tools such as imaging and laboratory testing are all necessary for proper diagnosis. Choosing the right lab tests and interpreting them correctly are critical components of Graves’ disease diagnosis and monitoring. A variety of thyroid antibody and hormone assays are currently available. Understanding the differences between the tests offered is important to ensure the right assay is chosen.

Results: TSI, which is the cause of Graves’ disease, can be detected in the blood of the majority of Graves’ patients. This important assay is often confused with a similar thyroid receptor antibody test called TRAb, which detects thyroid blocking antibodies in addition to the stimulating antibodies. The differences between these assays will be presented along with published data. The laboratory needs for proper Graves’ assessment will be discussed and why a fast, sensitive, and specific TSI assay is important through the continuum of Graves’ patient care.

Conclusion: In order to choose the right diagnostic tools for the assessment of Graves’ disease patient status, it is critical to understand the role of the various thyroid tests available. A fast, accurate diagnosis and careful monitoring is key to ensuring proper patient management.

The role of lactate in the assessment of mortality and morbidity

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Objectives:
- Understand the clinical importance of lactate, causes of elevated levels and its relevancy as an early indicator of septic infections
- Review the etiology of sepsis and its importance in morbidity and mortality
- Examine the variables and recommendations in current protocols for the management of sepsis
- Learn how whole blood lactate measurements can improve the outcome of septic patient survival rate

Background: Lactate is a byproduct of anaerobic metabolism. Elevated levels of lactate values are essential in identifying tissue hypoperfusion and patients that are at risk for septic shock. The rate of sepsis has doubled over the last decade and growth is projected to continue. Worldwide, 18 million sepsis cases occur annually and are estimated to increase 1.5% per year. In the United States, severe mortality rate is ~38%. The ability to quickly identify patients at risk of sepsis is critical to morbidity and mortality.

Methods: Quick detection of sepsis and rapid therapy is critical to patient outcome. For the best outcome, it is imperative that comprehensive information is available immediately and that the appropriate therapy is initiated. Lactate measurement is a readily available diagnostic test that can aid in the interpretation of sepsis and the determinant of survival.
Results: Data showing that lactate results can be a predictor of mortality will be presented. The rationale for the importance of this assay as a standard lab test and inclusion in therapy protocols will be reviewed. In addition, differences between whole blood and serum lactate tests will be discussed.

Conclusion: To ensure the best possible patient outcome, lactate measurement should be a standard of care in every medical institution. In the management of severe sepsis and septic shock, the rapid assessment of blood lactate should be implemented in early goal-directed therapeutic protocols and can be imperative in decreasing mortality.

WS5: Euroimmun AG

Molecular allergology: advanced diagnostics for a better quality of life

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About 30-40% of European population now suffers from at least one form of allergy. More of 50% of these allergic patients are polysensitized. Precise in-vitro diagnostics thereby complement conventional diagnostics. Immunoblots containing optimized combinations of relevant allergens provide efficient multiparameter analysis of specific IgE antibodies (sIgE) delivering a comprehensive and detailed patients profile in a single test. Molecular allergology also named component-resolved diagnostics (CRD) is an up to date approach to allergy diagnostics, whereby defined single alergen components are used for detection of sIgE in place of traditionally used alergen extracts. The molecular components are purified proteins, either isolated directly from the allergen source or produced recombinantly. CRD enables highly differentiated diagnostics discriminating cross reactions from multiple sensitizations, thus pinpointing the precise trigger of the allergy. Molecular allergy diagnostics is a powerful tool, facilitating risk assessment and therapy decisions, enabling the prevention of unnecessary burden to patients due to lifestyle changes or multiple therapies.

Plenary I

Personalized Cancer Therapy: Lessons From Laboratory Hematology

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In late 80 Cystic Fibrosis Foundation decided to found research on the cause of cystic fibrosis. As a result Kalydeco was produced which was the first FDA approved “targeted therapy”.

In cancer, personalized medicine uses specific information about a person’s tumor to help diagnose, plan treatment or make a prognosis. Integral part of personalized medicine is pharmacogenomics which help us to predict how the variability of the expression of genes between people leads to differences in susceptibility to disease and responses to medicines. In hematology single gene or sets of gene analysis provide us important information about thiopurine S-methyltransferase genetic polymorphism, which information is crucial for prediction of mercaptopurine or thioguanine metabolism. All those drugs are approved FDA with labeling regarding pharmacogenomic biomarkers. Nowadays we can use results of whole genome analysis to identify, for example, how inheritance affects methotrexate plasma disposition among children with ALL.

In September 1998, FDA approved Herceptin for the treatment of breast cancers which was the first genetically-guided therapy cancer. It was soon followed by approval for Rituximab which significantly improved outcome of treatment of patients with lymphoid malignancies. Now monoclonal antibodies are becoming integral part of the treatment of most blood cancers. Perspective of the new form of immunotherapy, like CAR-T cells looks very promising.

The best known hematological breakthrough drug is Glivec. Long time has passed since discover of chromosome Philadelphia to the introduction of the first tyrosine kinase inhibitor but now even mutated form of the enzyme can be effectively suppressed by specific inhibitor.

Personalized therapy is now facing economic challenges. Soliris (eculizumab), tailored monoclonal antibody produced to treat small group of patients with paroxysmal nocturnal hemoglobinuria, according to Forbes, at $409,500 a year, was the world’s single most expensive drug of the year 2009.
Plenary II

Translational Aspects of Inflammation in Atherosclerosis

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Chronic inflammation plays a well-established role in several cardiovascular pathologies, such as atherosclerosis, vascular calcification and aortic valve stenosis. Different inflammatory pathways could potentially be targeted for cardiovascular prevention. As an example, lipoxygenase metabolism of arachidonic acid yields the pro-inflammatory mediators leukotrienes. Targeting the specific leukotriene receptors reduces atherosclerosis and intimal hyperplasia in different animal models. In addition, anti-leukotrienes used in the treatment of asthma have been associated with decreased cardiovascular risk in observational studies, hence reinforcing the potent pro-inflammatory and pro-atherogenic role of this class of lipid mediators. In contrast, other lipoxygenase-derived lipid mediators act as “stop signals” for inflammation and induce a resolution of inflammation. The latter class of pro-resolving lipid mediators includes for example lipoxins and omega-3 fatty acid-derived resolvins, and stimulating their respective receptors may represent an alternative therapeutic strategy to target cardiovascular inflammation. In conclusion, lipid mediators may play a dual role in the non-resolving inflammation associated with atherosclerosis and valvular heart disease, and the balance between these mediators may have important implications for cardiovascular risk assessment and prevention.

Session 4: Laboratory assessment of kidney function

Novel Endogenous Metabolites for the Estimation of the Glomerular Filtration Rate (GFR)

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Creatinine is the most commonly used blood markers for the assessment of kidney function. A patient’s creatinine blood level is used alongside age, gender and race in validated equations to provide an estimate of GFR (eGFR). The use of age, gender and race as co-variates is warranted to account for non-glomerular determinants that affect the relationship between creatinine and the GFR. However, to date, there remains a significant performance gap between creatinine-based estimates of GFR and the mGFR, that is, GFR when measured by a gold standard methodology using a so-called ideal filtration marker. Today, the rate of large errors of current eGFR methodologies remains high with obvious implications for the management of patients across many clinical settings. Thus, novel markers of GFR like cystatin c have been sought with the goal to improve the precision and accuracy of GFR estimates. Recent advances in metabolomics technologies has enabled the survey of hundreds of small endogenous molecules (metabolites) in large sets of biological specimen, offering a new means to discover novel endogenous metabolite markers of kidney function. Several independent studies in diverse populations, in different clinical settings have yielded similar sets of candidate metabolites markers of GFR and recent verification studies are supporting the potential clinical value of metabolites like acetyl-threonine, pseudouridine and others for the precise and accurate estimate of GFR.

Cystatin C as a marker of kidney function in children

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Over the last two decades, cystatin C has evolved to be the most reliable endogenous parameter of kidney function and it is therefore included in many equations for GFR estimation both in adults and children.

The presentation will review the characteristics of cystatin C from a clinical point of view, focusing on its use in children.

Due to its molecular size, cystatin C does not cross the placenta allowing for assessment of renal function both in utero and directly post-partum, where creatinine fails due to equilibration with maternal values.

During the first year of life, cystatin C levels decrease reflecting maturation of GFR, which reaches adult values (if corrected for body surface area) by one year of age. While the reference range of creatinine increases with age reflecting increasing muscle mass during growth, this is not the case for cystatin C. In fact, adult reference data can be applied in children and cystatin c-based GFR estimation equations have been developed covering the entire age range from childhood to adulthood.

Specific indications where cystatin C has proven superior to creatinine in childhood are spina bifida/neuromuscular disease, patients with monofunctional kidneys and malignancy. In the latter group, drug dosing can be performed directly based on serum cystatin C.
Due to the smaller volume of distribution, cystatin C reflects changes in GFR more quickly than creatinine and has been shown to be an earlier indicator of acute renal injury.

Based on these characteristics it is not astonishing that GFR estimating equations incorporating cystatin C out-perform simple creatinine-based equations.

Cystatin C: Shrunken pore syndrome and death

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Cystatin C and creatinine are the two mostly used markers of GFR and the results should preferably be presented as estimates of GFR using so-called GFR-estimating equations. If the creatinine term is supplemented by terms for age, sex and race in such equations, the diagnostic performance of the creatinine-based equations is often comparable to that of cystatin C-based equations with cystatin C as the only term. However, one consistent difference between cystatin C- and creatinine-based equations is, that the estimate obtained by cystatin C-based equations is a much better marker for the development of cardiovascular manifestations, hospitalization, end-stage renal disease (ESRD), and death, than an estimate obtained by creatinine-based equations. The reason for this has been unknown, but it has recently been suggested that a new syndrome, “Shrunken Pore Syndrome, SPS”, might explain the better predictability of cystatin C for serious events (1). SPS is defined by that the estimation of GFR in an individual by a cystatin C-based equation is less than 70% of that of a creatinine-based equation (1, 2). In the two populations, investigated so far, the mortality increase in individuals suffering from SPS is very great, irrespective of whether the measured GFR is normal or decreased (2). This is most probably due to the altered signal peptide/protein patterns in individuals with SPS with an increase in the plasma levels of several 5-35 kDa peptides/proteins.


Short Communication

Cystatin C provides a better estimate of the effect of glomerular filtration rate (GFR) on serum HE4 concentrations

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Background: We evaluated the effect of kidney glomerular function on serum HE4 concentrations using creatinine, cystatin C and related Chronic Kidney Disease-Epidemiology Collaboration (CKD-EPI) equations.

Methods: We enrolled 101 women with a GFR (estimated by CKD-EPI eGFRCr) ranging from 60 to 120 mL/min/1.73m², free of ovarian cancer, any other evident disease and of biological and life-style factors known to influence serum HE4 concentrations, including age >60 years. In these subjects, we measured serum creatinine, cystatin C and HE4 concentrations by Abbott assays on Architect family platforms. Creatinine and cystatin C values were included in the three CKD-EPI equations to obtain GFR estimates.

Results: A statistically significant increase in HE4 median concentrations was detected in subjects with an eGFRCr between 60 and 74 mL/min/1.73m² when compared with those with an eGFRCr >90 mL/min/1.73m² (54.2 vs. 42.2 pmol/L, P = 0.003). Regression models showed that cystatin C measurement per se and eGFRCysC were the most sensitive markers to catch HE4 increases due to a mild decrease in renal function [adjusted r², 0.38 (P = 0.00003) and 0.37 (P = 0.0004), respectively]. By assuming baseline cystatin C and eGFRCysC at 0.80 mg/L and 101.5 mL/min/1.73m², an increase of 0.10 mg/L in cystatin C concentrations and a decrease of 10 mL/min of eGFRCysC implied an average (±SE) increase in serum HE4 concentrations of 9.2 (±1.2) and 8.8 (±1.1) pmol/L, respectively.

Conclusions: Our study shows that a better estimate of the effect of GFR on serum HE4 is obtained by measuring cystatin C in serum or using CKD-EPI eGFRCysC equation.
Session 5: Trends in pediatric laboratory medicine

Pediatric Obesity and Metabolic Syndrome

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Over the past few decades, there has been an alarming rise in incidence of childhood obesity and its metabolic complications worldwide leading to much higher rates of type 2 diabetes in children and adolescents. Insulin resistance (or “prediabetes”) is a central pathophysiological feature of type 2 diabetes and abdominal obesity, and is commonly associated with metabolic dyslipidemia. The current consensus is that obesity and insulin resistance may be part of a common pathologic state termed the “metabolic syndrome”. The metabolic syndrome, formerly referred to as insulin resistance syndrome or syndrome X, is characterized by a constellation of pathologies that include glucose intolerance, insulin resistance, obesity, dyslipidemia, and hypertension. Insulin resistance generally develops as the first indicator of type 2 diabetes and manifests as a decreased biological response to normal levels of circulating plasma insulin. Indicators of insulin resistance include impaired glucose tolerance, hyperglycaemia, and elevated plasma insulin levels. As long as the pancreas can compensate for the decreased insulin response by increasing insulin secretion, the individual is able to control blood glucose level. Allowed to continue untreated, however, the pancreas eventually fails to produce sufficient insulin, and type 2 diabetes occurs. Although not formerly considered a disease of childhood, type 2 diabetes has begun to present with increasing frequency in the paediatric population. It is feared that the disease progression begins early in life, and persistence from childhood to adulthood produces type 2 diabetes and cardiovascular disease in early adulthood.

The main objectives of this presentation are to review the pathophysiology of childhood obesity and metabolic syndrome, and the role of the clinical laboratory in the diagnosis and monitoring of children with insulin resistant states. A number of new and emerging laboratory biomarkers of insulin resistance and prediabetes, and their diagnostic value will be reviewed.

Expanded newborn screening for inborn errors of metabolism: the lysosomal diseases

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Background: There is an intensive discussion about the inclusion of LSDs on newborn screening panel. LSDs screening presents several challenges due to the nature of the conditions and lack of information regarding phenotypic spectrum, timing of treatment interventions, as well as a lack of professionally approved guidelines. We report our experience of LSDs screening for Fabry, Pompe, Gaucher and Mucopolysaccharidosis Type I (MPS I) diseasesin the North East of Italy.

Materials and methods: The enzyme activities of α-galactosidase A (GLA), α-glucosidase (GAA), β-glucocerebrosidase (ABG) and α-L-iduronidase (IDUA), were analyzed in dried blood spot (DBS) by stable isotope dilution flow injection analysis MS/MS (FIA-MS/MS) using the NeoLSD kit (Perkin Elmer). Infant with low activity (<0.25 ile) are recall for a second DBS. If low activity were confirmed, neonates were referred to our Unit for clinical assessment and further investigations.

Results: Of those 17365 newborns screened, 53 (0.31%) were recall for a second DBS, 13/53 (25%) underwent to confirmatory testing including clinical evaluation, enzyme assay in leucocytes/lymphocytes and mutational analysis. 4 newborns had a confirmed diagnosis of an LSD genotypewith a total incidence of 1 in 4341 births. The number of confirmed positive case corresponds to detection rates of 1: 8682 for Pompe disease,1:17365 for Gaucherand Fabry disease.5 newborns (3 with Fabry disease and 2 with MPS I) are currently classified as a genotype of unknown significance/onset and pseudodeficiency. Among the newborns who screened positive for GAA deficiency 1 was diagnosed with the infantile form and 1 with the late-onset disease.

Discussion. Our experience highlights some difficulties with result interpretation due to a lack of published information regarding phenotypic spectrum, timing of treatment interventions, as well as a lack of professionally approved guidelines. We report our experience of LSDs screening for Fabry, Pompe, Gaucher and Mucopolysaccharidosis Type I (MPS I) diseasesin the North East of Italy.

Expanded newborn screening for inborn errors of metabolism: the lysosomal diseases

Pediatric reference intervals: Challenges and recent advances

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For a proper diagnosis, monitoring therapies or establishing the prognosis of disease in children, results of clinical laboratory investigations probably play an even larger role than in adult patients. In the course of the physiologic development of a child from birth to adolescence,
dramatic changes occur resulting in significant modifications in metabolic pathways which are reflected in the concentrations of clinical laboratory parameters in the body fluids of these children. For a sound judgment of these laboratory results, it is thus indispensable to have access to reference intervals which are valid for the respective developmental stage of the pediatric patient under consideration. Ideally, reference interval upper and lower limits, expressed as a continuous function of the actual age of the child, would be required. In recent years, many studies dealing with thoroughly validated reference intervals for a large variety of clinical laboratory tests in children of all ages, using different approaches, have been published. Examples for studies performed in population-based investigations, studying large cohorts of healthy children in Europe, North America and some other countries will be presented. Furthermore, up-to-date information on patient-based reference intervals also obtained in a variety of countries will be presented and their advantages and disadvantages will be discussed. Furthermore, special aspects resulting from studies on pediatric reference intervals will be elucidated, such as premature born babies, preanalytical considerations, intra-individual variations of concentrations of biomarkers, and projects which have subsequently evolved from these studies.

Short Communications

Marked influence of BMI on biochemical markers of the metabolic syndrome in the CALIPER cohort of healthy children and adolescents

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Objectives: Reference intervals, essential to accurately interpret laboratory tests, are severely lacking for pediatrics, potentially causing misinterpretation. We present a sensitive and accurate method for the quantification of meropenem in human serum. TDM of meropenem can be used as an additional tool for clinicians to optimize dosing and to improve clinical outcome, allowing a tailored therapy.

Methods: Meropenem calibrators were prepared in blank human serum (2.0 - 200.0 µg/mL). Internal standard (IS) solution (25 µg/mL cefopazone), inflammatory markers (C3, C4, CRP, ALT), and nutritional markers (vitamin B12, vitamin D, ferritin) were measured. Biomarker levels were compared between normal weight (NW), overweight (OW) and obese (OB) subjects using one-way ANOVA and Bonferroni’s Post-Hoc test. Independent samples t-test compared NW and OW/OB.

Results: OW and OB adolescents had elevated triglycerides, apoB, C3, C4, and CRP and decreased vitamin B12 compared to NW. ALT and ferritin were elevated in OW/OB adolescent males, but not females. HDL-C was decreased in OW/OB adolescent males, but not females. Triglycerides and C3 were elevated in OW/OB compared to NW children.

Conclusions: Increased triglycerides and apoB, and decreased HDL-C in OW/OB adolescents suggest lipid abnormalities manifest prior to developing insulin resistance. Elevated inflammatory proteins suggest chronic low-grade inflammation in OW/OB children/adolescents. Lower vitamin B12 in OW/OB adolescents could result in hyperhomocysteinemia and increased CVD risk. Reference intervals for MetS biomarkers should either be partitioned by BMI or OW/OB subjects should be excluded if increased levels predict disease development.

Therapeutic drug monitoring of meropenem in pediatric laboratory medicine: a challenge

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Aim: Necrotizing enterocolitis continues to be a major cause of neonatal morbidity and mortality. Unfortunately, little information is available regarding the disposition of meropenem in neonates. Therefore, we want to highlight the emerging importance of therapeutic drug monitoring (TDM). We established and validated a HPLC-DAD method for quantification of meropenem in human serum.

Methods: Meropenem calibrators were prepared in blank human serum (2.0 - 200.0 µg/mL). Internal standard (IS) solution (25 µg/mL cefopazone) was prepared in CH3OH. For sample preparation, 200 µL serum and 400 µL IS solution were mixed. After centrifugation, the supernatant was evaporated under nitrogen. The residue was reconstituted (100 µL KH2PO4 buffer pH 3), and 20 µL was injected for HPLC analysis. Separation was performed on a Nucleosil C18P analytical column (Macherey – Nagel, Düren, Germany), using KH2PO4 buffer pH3 and 80% acetonitrile in a gradient system. The monitoring wavelength was 300 nm for meropenem and 290 nm for the IS.

Results: Total run-time including column re-equilibration time was 30 min. Intra- and inter-run precision (n=6) were < 10 % and < 15 % respectively for 4 QC levels. Accuracy (expressed as relative error) was < 10 %. The assay was linear from 2.0 to 200 µg/mL (mean r2=0.9995, n=6). Quantification limit was 2.0 µg/mL.

Conclusions: We present a sensitive and accurate method for the quantification of meropenem in human serum. TDM of meropenem can be used as an additional tool for clinicians to optimize dosing and to improve clinical outcome, allowing a tailored therapy.
Session 6: WASPaLM: Molecular Diagnostics in Cancer Management

Circulating tumor DNA – a promising diagnostic tool in cancer

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High-quality genomic analysis is critical for a personalized medicine approach to cancer patient management. Genome sequencing can potentially identify the molecular abnormalities that predict either good or poor outcomes and to identify new targets for therapy. Higher rates of point mutations and chromosomal aberrations were found, for example, in aromatase inhibitor-resistant ER+ breast cancers. Tumor-specific genomic alterations identified in cell-free DNA (cfDNA) from patient blood samples seem to be helpful to monitor relapse or response to treatments. In patients with metastatic breast cancer, circulating tumor DNA showed a better correlation with tumor burden and was an earlier measure of treatment response compared to both CA 15-3 and circulating tumor cells. Non-invasive analysis of acquired resistance to cancer therapy is possible by detection of somatic mutations in plasma cfDNA and allows for the identification of specific mutations selected by treatment such as \textit{EGFR T790M} in patients with NSCLC treated with gefitinib. Gains and losses of chromosomal regions have been detected in plasma tumor-specific cfDNA as copy number aberrations and can be used to compute a genomic copy number instability index (CNI) of cfDNA. The CNI obtained by massive parallel sequencing discriminated prostate cancer from controls, and benign prostatic disease. CNI change may also serve as a potentially early predictor of response to standard chemotherapy for various cancer types (e.g. NSCLC, colorectal cancer, pancreatic ductal adenocarcinomas). Cancer diagnostics and therapy monitoring is one of the most relevant and fastest growing areas of clinical diagnostics.

Next-generation sequencing: where we are and where we are going

David S. Wilkinson
Department of Pathology, Virginia Commonwealth University School of Medicine, Richmond, Virginia, USA

Next-generation DNA sequencing technology (NGS), also known as massively parallel sequencing, is revolutionizing cancer genomic diagnostics, enabling precision cancer medicine by directing molecularly targeted therapies. This presentation will provide a brief history and overview of the key chemistries used for NGS, as well as the instrument platforms available for use in the clinical laboratory.

Biomarker tests for molecularly targeted therapies: key to unlocking precision medicine

Andrea Ferreira-Gonzalez
Division of Molecular Diagnostics, Department of Pathology, Virginia Commonwealth University, USA

The utility of targeted next-generation sequencing-based assays for identification of genomic variants has made significant advances in the field of molecular oncology. Testing panels and platforms have enabled clinical molecular pathology laboratories to expand their testing menu to include NGS assays that can provide information for a more reliable prediction of personalized cancer therapies. Additionally, the assays can identify relevant genes that may have implications for enrollment of the patient in clinical trials. This presentation will focus on the issues involved for optimal performance and implementation characteristics of assays for the identification of somatic variants in solid tumors in an academic molecular pathology laboratory.

Short Communications

Molecular diagnostic of hepatocellular carcinoma using cell-free DNA

Elodie Lebredonchel
Genopathies, Lille Hospital, France

Hepatocellular carcinoma (HCC) is the sixth most prevalent cancer and the second most common cause of cancer-associated death worldwide, with a poor prognosis. Few therapeutic strategies have been proven efficient, it is vital to find new biomarkers for early diagnosis. Cell-free DNA (cfDNA) study or “liquid biopsy” is expected to be a useful candidate in an emerging class of blood tests to replace repetitive tissue biopsies and improve the patient comfort, obtain diagnostic, prognostic, and theranostic information for cancer, especially HCC.
In this study, we targeted and sequenced four regions of interest by Mi-seq next-generation sequencing (NGS) from 14 patients samples. This provided data about two mutations and two methylation hotspots.

TERT (Human telomerase reverse transcriptase) is mutated up to 59% in human hepatocellular carcinoma. To our knowledge, this is the first evidence linking mutations in the TERT promoter and HCC. Moreover, the Wnt/ßcatenin pathway is a major player in the development of HCC and previous studies. CTNNB1 codes for ßcatenin and is mutated up to 26% in human liver tumor HepG2 cell line and had to be explored in priority.

To investigate methylation status we selected 2 hotspots candidates CELF6 and RNF135 containing CpG islands that can be methylated in HCC process. To improve the current NGS library preparation protocol we had to include bisulfite PCR.

CTNNB1 study gave the same results on CnDNA as on tissue biopsies, the others were promising. Further investigations will be PCR improvement, comparison with healthy volunteers and with the full 267 patients’ cohort.

The role of selectins (SP-, SL-, and SE-) in the progression of colorectal cancer

Violetta Dymicka-Piekarska, Aleksandra Korniluk, Paweł Kiszło, Halina Kemona
Department of Clinical Laboratory Diagnostics, Medical University of Białystok, Poland

Introduction/Aim: The aim of this study was to evaluate the potential association between the level of soluble selectins (P-, L-, E-) in patients with colorectal cancer and the severity of the disease. Therefore, the assessment its diagnostic utility will be done.

Material and methods: The study included 53 patients with colorectal cancer (CRC) (29 men, 24 women, mean age 66.9) and in 25 healthy subjects. The patients were divided into three groups according to TNM classification. Soluble levels of P-, L- and E-selectin were measured in plasma using commercial enzyme immunoassay kits (R&D Systems).

Results: Mean levels of sP-, sL- and sE-selectins in all CRC patients were significantly higher than in healthy subjects (p < 0.001). The highest level of sP-selectin was observe in patients with metastases to the liver and was significantly higher than in patients without metastases (p < 0.01) and with lymph node metastases (p < 0.01). The level of sL-selectin, same as sE-selectin, was the highest in patients with lymph node metastases. The greatest area under the ROC curve (AUC) was for sP-selectin (0.881, p < 0.001), which indicate its good diagnostic power.

Conclusion: Among those selectin, P- selectin plays an important role in the progression of colorectal cancer and could be an attractive marker with clinical significance.

Debate I: Vitamin D: To test or not to test?

Pro:

Roger Bouillon, University Hospital, Catholic University of Leuven (B)

No abstract available

Contra:

Ian Young, Department of Medicine, Queen's University, Belfast (UK)

No abstract available

WS6: EFLM-UEMS workshop: Putting patient focused laboratory medicine into practice

Is there Support for Patient Focused Laboratory Medicine in Europe?

Ian D. Watson
EFLM Working Group on Patient Focused Laboratory Medicine c/o EFLM Office, Via Carlo Farini 81, 20159 Milano, Italy

Technological change has driven the so-called ‘Age of Knowledge’; this applies in Medicine as elsewhere. Practice varies across Europe on patients’ access to their medical record and to laboratory test results, in some countries there is ready access and in others there are legal restrictions. Even where there is access, there is rarely individualised explanation of test results unless through the requesting physician.
To understand results patients may access definitive sources such as ‘Lab Tests OnLine’, but this is not individualised. With increasing expectations by patients and healthcare systems that patients have responsibilities in managing their health along with healthcare professionals that if they wished individualised support this may well overburden their physician. Particularly in the area of chronic disease management, but not necessarily exclusively, there may be a role for other healthcare professionals. We determined to assess the level of support amongst patients and Specialists in Laboratory Medicine for the latter to provide the support proposed to patients.

We surveyed seven countries and in all there was majority support from patients for such a proposal and there was also majority support from the profession across Europe for such provision. Understandably there were variations between countries e.g. legislation impeding such a service; patients preferring any advice to be free rather that paid for. Options for providing such support to patients will be considered.

**Patient-focused laboratory medicine: Horizon 2020 projects**

Wytze P. Oosterhuis  
Zuyderland Medical Center, Department of Clinical Chemistry and Haematology, Heerlen, The Netherlands; EFLM Working Group of Patient Focused Laboratory Medicine.

There is a growing demand from patients to be better informed and participate more actively in treatment decisions. Initiatives to give patients access to their data, such as patient portals are a reflection of this development. Better informed patients are better equipped to participate in the medical decision process. The terms patient empowerment and shared decision making are often used in this context. It has been shown that a better involvement of patients leads to an improved motivation to adhere to treatment, with a better health outcome. Many physicians are however concerned that record access will create more work, with extra consultations and telephone calls as a result of patients’ misunderstandings. Studies suggest the opposite – that record access can reduce resource demand. Both the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) and the Section of Laboratory Medicine of the UEMS (Union Européenne des Médecins Spécialistes) recognize the development of patient empowerment and the changing role of care providers and patients. To this end, the EFLM has established the Working Group Patient Focused Laboratory Medicine, that took the initiative to apply for funding within the framework of the EU Horizon 2020 programme.

The overall aim of this initiative is to design, develop and evaluate an interpretative knowledge and reporting system that will inform patients so that they can comprehend the significance of their laboratory test results, enabling them to better participate in the shared decision process with their physician. The system represents a new integrated approach to process the data from other healthcare systems and communicate the results in a flexible way to different receiving systems and individual using several advanced types of communication.

**WS7: EFLM workshop: Cardiac Markers today**

**Evidence based cardiac biomarker testing – guidelines or guesswork**

Paul Collinson  
St George’s Hospital, London on behalf of the Working group – Cardiac Markers of EFLM.

Background. To assess current use of evidence-based guidelines for the use of cardiac biomarkers in Europe and North America.  
Methods. In 2006 and 2010 a web-based questionnaire was distributed via European biochemical societies and in 2013/14 this included a pilot in North America. Questions covered cardiac biomarkers measured, analytical methods used, decision thresholds and use of decision-making protocols. Results were collated using a central database and analysed using comparative and descriptive nonparametric statistics.  
Results. In the most recent survey, in Europe (EU), returns were obtained from 442 hospitals, 50% Central or University hospitals and 39% from local hospitals from 35 countries. 395/442 (89%) provided an acute service. In North America (NA) there were 91 responses (63.7% Central or University hospitals, 19.8% community hospitals) with 76/91 (83.5%) providing an acute service. Cardiac troponin was the preferred cardiac biomarker in 99.5% (EU) and 98.7% (NA) and the first line marker in 97.7% (EU) and 97.4% (NA). There were significant differences in the choice of decision limits and their derivations.  
Conclusions. Although cardiac troponin is the dominant biomarker, other markers are still retained. There is a significant failure to use the evidence based cut offs for cardiac troponin. Differences between European and North American practices probably relates availability of assays and protocols for assay introduction.
Natriuretic peptide testing in Europe (and beyond)

Angelika Hammerer-Lercher1, Paul Collinson2, Janne Savisaari3, Fred S. Apple4, Rob H. Christiansen5, Kari Pulkki6, Marja P van Dieijen-Visser7, Christopher J. Duff8, Hannsjörg Baum9, Ana Stavljenic-Rukavina10, Kristin M. Aakre11, Michel R. Langlois12, Sanja Stankovic13, Paivi Laitinen10 on behalf of the Working Group for Cardiac Markers (WG-CM), European Federation of Clinical Chemistry and Laboratory Medicine (EFLM).

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9 Regionale Kliniken Holding RKH GmbH, Germany
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13 Center for Medical Biochemistry, Clinical Center of Serbia, Belgrade, Serbia

Background: The aim of this survey was to investigate how well heart failure (HF) guidelines for the use of natriuretic peptides (NP) have been implemented in laboratory practice in Europe and North America.

Methods: In 2013/14 a web-based questionnaire was distributed via North American and European biochemical societies. Questions covered type of assay performed, reason for method choice, decision limits for HF and laboratory accreditation status.

Results: There were 442 European and 91 North American participating laboratories with returns from Central or University hospitals in 50% and 64% of returns, respectively. NP measurements were offered in 67% of European and 58% of North American respondents. NT-proBNP was most widely used in Europe (68%) compared to BNP’s use being more (58%) in North America. The most frequent reason was the availability of instruments, which measure either NT-proBNP or BNP; 51% and 67%, respectively. For acute HF, NT-proBNP decision limits were very diverse and the age dependent limits based on the 2012 ESC recommendations were used only 17% in Europe and 26% in North America. Concerning BNP, the guideline recommended acute HF decision limit of 100 ng/L was better adhered to in Europe, 48% and North America, 67%. Surprisingly, similar decision limits were offered for chronic HF as for acute HF.

Conclusion: NP measurement for HF diagnosis was available in more than half of the responding laboratories. However, guideline recommended cut-off values for both and chronic HF were still not adequately implemented.

Friday, September 23rd

Parallel sessions

Session 7: Dyslipidemia: New clinical concepts and diagnostic tools

Triglycerides, remnant cholesterol and lipoprotein(a) for cardiovascular risk prediction

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New insight in epidemiology now suggests that triglyceride-rich lipoproteins, marked by high triglycerides, are strong and independent predictors of cardiovascular disease and all-cause mortality, and that their cholesterol content or remnant cholesterol likewise are strong predictors of cardiovascular disease. Of all adults, 27% have triglycerides above 2 mmol/L (176 mg/dL) and 21% have remnant cholesterol above 1 mmol/L (39 mg/dL). For individuals in the general population with nonfasting triglycerides of 6.6 mmol/L (580 mg/dL) compared with individuals with levels of 0.8 mmol/L (70 mg/dL), the risks were 5.1-fold for myocardial infarction, 3.2-fold for ischemic heart disease, 3.2-fold for ischemic stroke, and 2.2-fold for all-cause mortality. Also, genetic studies using the Mendelian randomization design, an approach that minimizes problems with confounding and reverse causation, now demonstrate that triglyceride-rich lipoproteins are causally associated with atherosclerotic cardiovascular disease and all-cause mortality.

Human epidemiologic and genetic evidence using the Mendelian randomization approach in large scale studies likewise now strongly support that elevated lipoprotein(a) is a causal risk factor for cardiovascular disease, that is, atherosclerotic stenosis, myocardial infarction, and aortic valve stenosis.

Taken together, new insights now strongly suggest that elevated triglyceride-rich lipoproteins and elevated lipoprotein(a) represent causal risk factors for cardiovascular disease.
Joint EFLM-EAS Consensus Guidelines on Non-fasting Dyslipidemia Testing

Michel R. Langlois
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Chair of EFLM Task & Finish Group on Laboratory Testing for Dyslipidemia (TFG-LTD)

Fasting samples have been the standard for lipid measurements, despite the fact that we spend the vast majority of our time in nonfasting conditions. However, if postprandial effects do not substantially alter lipid levels or their association with cardiovascular risk, then a nonfasting blood draw has many practical advantages. Studies of the European Atherosclerosis Society (EAS) and European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) Consensus Panel suggest that postprandial effects do not weaken, and even may strengthen, the risk associations of lipids with cardiovascular disease.

Research from Denmark, Canada and the US involving more than 300,000 individuals showed that most lipid measurements differ minimally when performed nonfasting or fasting, with only modest increases (up to 25 mg/dL) for triglycerides, and consistently found either similar - or sometimes even stronger - cardiovascular risk associations for nonfasting vs. fasting lipids.

Nonfasting cholesterol measurements include the ‘remnant cholesterol’ fraction, a strong and direct causal risk factor for developing atherosclerosis independent of LDL cholesterol. ‘Remnant cholesterol’ is the cholesterol in VLDL- and chylomicron remnant particles; it is included in the calculation of ‘non-HDL cholesterol’ (= total – HDL cholesterol). Most cardiovascular prevention guidelines focused on targeting primarily LDL cholesterol, but they now recognize that non-HDL cholesterol (or apolipoprotein B, the molecule carried by non-HDL particles) is a more comprehensive predictor of risk.

The 2016 EAS-EFLM consensus is the first international recommendation to use nonfasting lipid tests for routine clinical practice and provided specific cutpoints for desirable fasting and nonfasting lipid concentrations to be reported by the laboratories uniformly. The time has come to move this strong evidence into action.

Apolipoprotein C-II: Recent clinical and basic findings

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Apolipoprotein (Apo) C-II is a small exchangeable apolipoprotein in plasma and is known to activate lipoprotein lipase, a key enzyme in the hydrolysis of triglycerides on lipoproteins for energy metabolism. Patients with a mutation of apoC-II typically present with elevated triglycerides. During this talk, we will first review the different genetic causes of hypertriglyceridemia and its potential clinical manifestation, such as pancreatitis and cardiovascular disease. Next clinical laboratory testing of hypertriglyceridemia will be discussed plus the development of new therapies for hypertriglyceridemia. The phenotype of a novel apoC-II knockout mice with elevated triglycerides plus obesity and insulin resistance will be shown. Finally, a new synthetic peptide mimetic of ApoC-II that activates lipoprotein lipase was developed and shown to correct the lipid abnormalities in the apoC-II knockout mice. The apoC-II mimetic peptide also improved insulin resistance in diabetic mouse models and markedly lowered post-prandial triglycerides in mice gavaged with olive oil, suggesting that this peptide may have more general use in the treatment of dyslipidemia. In summary, the clinical manifestations and laboratory diagnosis of apoC-II deficiency will be reviewed along with the other causes of hypertriglyceridemia. In addition, new basic science findings that lead to the development of a novel apoC-II mimetic peptide that may have utility beyond the treatment of apoC-II deficiency will be discussed.

Short Communications

LDL and HDL subfractionation - new clinical concept in diagnosis of dyslipidaemia in the eastern Slovakian majority and Roma population

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Lipoprotein subfractionation provides more detailed knowledge on quantity and on quality of the lipoproteins. Novel techniques allow determination of seven LDL and ten HDL sub-fractions, referring to the atherogenicity of small dense fractions. The subfractionation was carried out both in the majority population (M) as well as in Roma (R) with dyslipidemia, in order to identify differences in two ethnic groups.

Adult overweight dyslipidaemic (O = 40, BMI = 29.8 ± 3.5) and normal weight normolipidaemic (NW = 31, BMI = 22.3 ± 1.9) R, as well as O (59, BMI = 29.3 ± 2.9) and NW (40, BMI = 21.8 ± 2.3) M of the HepaMeta project participated in our study. The analysis of lipoprotein fractions was carried out using the Quantimetrix Lipoprint System. The parameters of serum lipid profile (total cholesterol, LDL-cholesterol,
HDL-cholesterol and triacylglycerols) were determined by routine biochemical methods. AIP and BMI were calculated as follows: AIP = log (triacylglycerols / HDL-cholesterol); BMI = body weight [kg] / squared height [m2].

Between group means showed no significant difference in serum lipid parameters between O and NW M compared to R, but in non-atherogenic HDL-3 (p = 0.010), HDL-6 (p = 0.001), HDL-7 (p = 0.004) and atherogenic HDL-8 (p = 0.004), HDL-9 (p = 0.019) and HDL-10 (p = 0.026) in OM compared to OR.

The present results pointed to an importance of the quantification of HDL sub-fractions, which is in the background of LDL fractionation in diagnosing dyslipidaemia in clinical practice, particularly with respect to ethnicity.

The study was supported by VEGA 1/0115/14 and KEGA 013 UPJS-4/2016.

New and old formulas for the calculation of LDL-cholesterol- an evaluation

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LDL-Cholesterol (LDL-C) is widely used for cardiovascular disease (CVD) risk assessment. The gold standard for measurement of LDL-C is by ultracentrifugation and beta-quantification. This is expensive and inconvenient for the routine laboratory. Other methods include direct measurement of LDL-C. Direct methods show poor performance with high triglyceride (TG) levels. Earlier reviews comparing direct measurement of LDL-C vs calculation of LDL recommended the use of direct LDL measurements in hypertriglyceridemic patients. However, a recent study comparing eight direct measurements of LDL-C and HDL-C failed to show improved CVD risk classification of most direct methods over calculated LDL-C.

The first formula to calculate LDL-C was developed over 40 years ago by Friedewald. The formula requires fasting plasma high density lipoprotein-cholesterol (HDL-C), total cholesterol (TC), and TG. This formula is less accurate in extremes of TG or TC values or in patients with co-morbidities (eg. renal failure or diabetes), but is widely used. Several other formulae have been developed, but these do not perform better than Friedewald’s calculation or have varying results in different population groups and when using TG ratios.

We have evaluated over 10 000 lipid profiles using a number of different formulae (Friedewald, Chen, de Cordova, Hattori) and have compared these to direct measurement of LDL-C across various triglyceride (TG), total cholesterol (TC) and HDL-cholesterol (HDL-C) ranges using Beckman reagents and instruments. Linear regression and ROC analysis were performed.

The Hattori formula outperformed all formulae including Friedewald over various ranges of lipid values.

In view of this and other evidence, we suggest that the use of the Friedewald equation be re-considered when evaluating cardiovascular risk.

NMR lipoprotein phenotyping for assessing cardiovascular disease risk

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Introduction: In routine practice, the standard lipid panel may not identify everyone at risk for cardiovascular disease (CVD). Nuclear magnetic resonance (NMR) lipoprotein testing allows one to examine in more detail lipoprotein particle number and size and has been proposed as an alternative CVD risk marker.

Aim of the study: The main purpose of this study was to develop a phenotypic classification for lipoproteins based on NMR analysis.

Methods: We used a set of over 22,000 plasma sample results that were run on the Vantera® Clinical NMR analyzer to develop 6 lipoprotein phenotypes (1, 2a, 2b, 3a, 3b, 4). The Vantera® provides a measurement of lipoprotein particle number and size and generates up to 21 results per sample from the raw NMR spectrum. To transform a group of related variables into a series of composite variables, we used principal component analysis (PCA). K-means clustering was then used for assigning the lipoprotein phenotypes.

Results: Using clinical endpoints of CVD disease and imaging data by CT-calcium scores from the MESA study, we found a strong association between the NMR-lipoprotein phenotype and CVD. Features strongly linked to CVD disease were high levels of LDL-particle number, particularly small LDL subfractions and low HDL-particle number. After adjustment for conventional CVD risk markers, the NMR-lipoprotein phenotype was still associated with CVD risk and showed a net reclassification score of approximately 6% for CVD events in MESA.

Conclusion: NMR lipoprotein phenotyping may be highly useful in conjunction with standard lipid testing for predicting CVD risk.
Session 8: New diagnostic tools for infectious diseases

Blood culture independent sepsis diagnostics

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Blood culture (BC) still is the gold standard for microbiological sepsis diagnosis. However, this method lacks sensitivity, especially for slow-growing or fastidious microorganisms and fungi. Substantial time delays due to impaired growth kinetics of blood microorganisms after antimicrobial treatment, low initial pathogen load, or volume of specimen inoculated, are observed frequently. Numerous non-infectious conditions can mimic sepsis, and the rapid detection of the causative pathogen is of particular importance since therapy and outcome differ greatly between patients with sepsis and those with non-infectious conditions. To meet the need for faster microbiological diagnostics, other methods have been introduced in routine clinical laboratories including PCR based tests for the molecular detection of microorganisms directly from whole blood. Most of these tests consist of multiple amplification reactions. Pathogen identification to the species level is performed by sequence specific fluorescent probes, BLAST analysis of the amplicon sequence, mass spectrometric amplicon analysis or gel electrophoresis. Some of these tests also allow for the detection of relevant resistance markers. Considering the advantages and limitations of these tests one may conclude that although they cannot yet replace BC, they may represent a valuable add on to culture-based diagnosis.

Overview: Laboratory diagnosis of Lyme borreliosis

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Despite significant progress in the diagnostics of Lyme borreliosis, including molecular methods, the detection of a specific antibody response still remains the mainstay in the laboratory diagnosis of the disease. Many current European guidelines propose the combination of highly sensitive screening assays, such as ELISAs, with very specific confirmatory tests, such as immunoblots, to guarantee a cost-effective, sensitive and specific diagnostic approach. This approach however tends to be increasingly questioned at least in the American setting where single tests or two EIA strategies are more and more propagated. Although all kinds of fully automated immunological test variants and cellular assays have been developed and pushed into the markets laboratory based Lyme borreliosis diagnostics tend to remain an art as there is no “one fits all strategy” and the investigator must always consider a whole series of clinical and laboratory facts for a correct interpretation of the serological findings. Here, we summarize current laboratory algorithms in the diagnosis of Lyme borreliosis, with a special emphasis on new or recent diagnostic developments and how to interpret such tests correctly in the context of additional clinical and laboratory information.

Update: Diagnostics and treatment of Hepatitis C

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The diagnostic criterion for chronic HCV-related disease is the presence of HCV RNA in blood serum, liver tissue or peripheral blood mononuclears, that sustained for at least six months. The virus may be responsible for both liver disease or an extrahepatic manifestation of infection. Chronic HCV infections may take the form of chronic hepatitis C, that may proceed to liver cirrhosis and hepatocellular carcinoma. Non-invasive methods of liver fibrosis assessment have been developed recently with predominance of elastography. Liver biopsy is used mostly when any discrepancy is observed and/or aetiology of liver disease is doubtful. The selection of treatment regimen should involve determination of the virus genotype and assessment of the stage of liver fibrosis. Therapy should be monitored by assaying the concentration of HCV RNA by techniques ensuring that the limit of detection is below 15 IU/ml for qualitative assessment, and does not exceed 25 IU/ml for quantitative assessment. In case of treatment failure assessment of Resistance Associated Variants (RAVS) may be of clinical significance. Introduction of direct acting antivirals (DAA) made kind of revolution in HCV infection treatment. Efficacy up to 95%, perfect safety profile and minimal contraindication let physician to treat almost all patients. Regimens based on pegylated interferon alfa are still used in Genotype 3 infection. Drug-drug interactions are main concern and specialist care is necessary.
Short Communications

Diagnostic utility of CXCL9 index in patients with tick-borne encephalitis

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Introduction and aim: Tick-borne encephalitis (TBE) is the most common tick-borne disease in Europe. The aim of the study was the evaluation of cerebrospinal fluid (CSF) and serum CXCL9 concentrations and diagnostic usefulness of this molecule in TBE. To exclude possible impairment of the blood-CSF barrier and/or blood brain barrier functions as a potential sources of the alterations in CXCL9 concentrations, the CSF CXCL9 concentrations were related to the concentrations in serum (ICXCL9).

Methods: The study included 24 TBE patients in acute phase (TBE I), and after 2 weeks follow-up (TBE II). The control group (N=13) consisted of patients investigated for suspected central nervous system infection (CNS), but with normal CSF findings. CXCL9 concentrations were measured using ELISA method. Differences were considered statistically significant for P<0.05. Receiver operator characteristic (ROC) curve was generated to calculate the area under the ROC curve (AUC).

Results: ICXCL9 in TBE I and TBE II were significantly higher as compared to the controls, and – in opposite to the CSF or serum CXCL9 concentrations – significantly decreased after 2 weeks. CSF CXCL9 AUC was higher than the AUCs for serum CXCL9 and ICXCL9. However all AUCs were very high (≥0.94) and significantly higher than AUC=0.500.

Conclusion: The evaluation of CXCL9 revealed diagnostic significance in distinguishing patients with TBE from subjects with initially suspected but later excluded CNS infection. However further studies are required to explain whether this protein might be utilized as potential tools for the diagnosis and monitoring of inflammation in TBE.

The role of digital PCR in the molecular diagnosis of infectious diseases

Jim Huggett, Carole Foy, Helen Parkes
Molecular and Cell Biology, LGC, United Kingdom

Digital PCR (dPCR) is a method that counts individual DNA molecules which has the potential to be far more sensitive and reproducible than contemporary methods such as quantitative PCR. The aim of this study was to describe the accuracy, sensitivity and reproducibility of dPCR when measuring a range of human pathogens. dPCR was applied to the quantification of human pathogens (including human cytomegalovirus, influenza and Mycobacterium tuberculosis) using a variety of clinically applied and newly developed assays. Technical performance was determine when measuring purified nucleic acids, as well when incorporating the extraction steps using reference materials in a variety of matrices. Reproducibility was evaluated by comparing the performance of different assays, instruments and laboratories from across Europe. dPCR performs was able to quantify pathogen nucleic acid with comparable precision to qPCR and was able to perform with high reproducibility without a calibration curve. Choice of extraction method influenced the quantitative bias as some methods did not recover the same nucleic acid yield, however with optimum extraction inter laboratory quantitative reproducibility remained high. dPCR also performed with higher sensitivity when measuring rare genetic variants, such as the single nucleotide variant associated with oseltamivir resistant influenza. Our findings demonstrate that dPCR offers high reproducibility suggesting it has the potential to act as a reference method to support established molecular methods like qPCR. As dPCR is developed the high reproducibility make it a potentially valuable diagnostic method and improved sensitivity potentially enable it to detect resistance at an earlier stage than conventional methods.

Session 9: Diagnosis of autoimmune disease

The mosaic of autoimmunity; The role of genetics and environmental factors and especially diet

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Zabludowitcs Center for Autoimmune Diseases, Sheba Medical Center.
Incumbent of the Laura Schwartz Kipp chair for Autoimmune diseases. Tel Aviv University, Israel

Autoimmune diseases are conditions in which the immune system damages normal components of the individual. Thus autoantibodies productions were found to be multifactorial in their etiology. For practical reasons these factors are classified into four categories:

- Genetic, which entail the MHC class I, II, and III. A case in point will be the haplotypes of HLA-DRB1 which are prevalent in many classical diseases.
- Immune deficiencies: C1q C2, C4 and IgA deficiencies are among the most common defects associated with diverse autoimmune conditions.
Hormonal state, most autoimmune diseases are detected in females at the child bearing ages. The role of estrogens will be delineated. In addition other hormones play a role i.e. prolactin.

Emergence environmental causes: Those are the most important as a trigger factors (i.e. adjuvants) determining the time and type of disease. They entail infectious agents, chemicals, drugs and even vaccines.

The type of disease in an individual, in an autoimmune prone family, will be determined by the specific combination of the different factors mentioned above.

A special emphasis will be put on smoking, on unsaturated fats, salty diet, chocolate, coffee, spicy food, cannabinoids, and the interaction with component of parasites.

References:

Hemophagocytic lymphohistiocytosis and macrophage activation syndrome

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Hemophagocytic syndrome, also known as hemophagocytic lymphohistiocytosis (HLH), is a severe, life-threatening inflammation caused by an excessive, prolonged, and ineffective immune response.

HLH can be divided into genetic (primary) and acquired (secondary) forms. An acquired HLH develops as a consequence of intense immune activation caused by: infection (I-HLH), autoimmune disease (A-HLH / MAS) or malignancy (M-HLH). HLH may also occur in the course of metabolic diseases (Gaucher disease, lizynuric protein intolerance), immunosuppressive therapy, as well as after organ and stem cell transplantation.

Among genetic HLH, familial HLH (FHLH) and other genetically determined forms are distinguished. Mutations in PRF1 (FHLH-2), UNC13D (FHLH-3), STX11 (FHLH-4) and STXBP2 (FHLH-5) cause different subtypes of FHLH. Hemophagocytic syndrome may occur in very rare immune deficiency syndromes running with albinism (Griscelli syndrome 2, Chédiak-Higashi, and Hermansky-Pudlak type II) and lymphoproliferative syndromes associated with chromosome X (XLP1 and XLP2).

Aim of the study: We analyzed genetics and clinical outcome of primary HLH and MAS among Polish pediatric population.

Methods: Forty five patients fulfilling the HLH-2004 criteria, treated at 12 Polish pediatric hematology/oncology centers, were included in the study. According to clinical indications, molecular analysis of PRF1, UNC13D, STXBP2, SH2D1A and BIRC4 was performed with the use of direct DNA sequencing.

Results: Genetic background of HLH was identified in 8/45 patients. One patient had SH2D1A nonsense mutation (Arg55X), consistent with XLP1 diagnosis, and one BIRC4 missense mutation (Glu219Lys) - XLP2 diagnosis. Six out of eight patients from 7 families had UNC13D mutations. 10 patients were diagnosed with MAS.

Choosing wisely: What is rational in autoantibody testing?

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The traditional role of autoantibodies testing is the discrimination of an autoimmune disease from non-autoimmune disorders. Thus, they are included in classification criteria of systemic and organ-specific autoimmune diseases being helpful in diagnosis, especially at early stages of the disease with oligosymptomatic manifestations or in patients with an “atypical” clinical presentation. Sometimes they might be also applicable to monitor the activity of the disease or to assess the prognosis. Of note, autoantibodies are not “gold standard” tests but a part of a diagnostic panel, and request for autoantibodies screening is justified when supported by clinical setting, defined by particular patient’s history and physical exam findings. Moreover, one has to consider many factors that influence serological diagnostics and make it unique and incomparrable to other fields of laboratory medicine. Biological heterogeneity of the autoimmune response, intra- and inter-population variations, a broad spectrum of autoantigens in one disease entity, the pre-symptomatic production of autoantibodies, strong interdependence between diagnostic sensitivity and specificity as well as methodological limitations, are only the selected aspects which may influence
interpretation and clinical relevance of serological test results. Additionally, novel autoantibodies are being constantly introduced which together with the better understanding of their pathogenic role are very welcomed, but also put forward some challenges. Despite all those aspects, serological tests when used properly provide valuable non-invasive way of diagnosing and monitoring disease. Further optimizing of autoantibodies testing, based on international efforts for standardization of multiparametric assays and harmonization of nomenclature and testing algorithms, requires close collaboration between clinicians, laboratory medicine specialists and diagnostic industry.

Short Communications

Diagnosis of Graves disease: performances of a novel fully automated assay with improved specificity for TSH stimulating immunoglobulins

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Background: Measurement of TSH receptor autoantibodies (TRAb) is important for the diagnosis and monitoring of Graves’ disease (GD). Several automated methods for testing are available but are not yet standardized and not specific of TSH receptor stimulating immunoglobulin (TSI).

Methods: We evaluated the IMMULITE® TSI assay (Siemens), a fully automated immunoassay based on the chimeric receptor that specifically binds TSI but not blocking autoantibodies. Assay’s imprecision was assessed with five pools of serum samples and with two levels of control materials. Reference values were determined with samples from 90 male healthy volunteers free of thyroid diseases and medications. Method comparison was performed with a second-generation TRAb enzyme immunoassay Medizym®.

Results: Between run coefficients of variation were 6.5 and 4.7% for concentrations of 1.0 and 22.6 IU/L, respectively. The limit of quantification of the IMMULITE assay, determined with the precision profile built with the 5 pools of serum, was below 0.1 IU/L. The concordance correlation coefficient between the TSI and TRAb assays was 0.82. The TSI levels measured with the IMMULITE assay in healthy volunteers were below 0.10 IU/L. The receiving operator curve analysis of patients with active Graves disease patients with other thyroid disorders and healthy controls revealed an AUC of 0.99 resulting in a sensitivity of 100% and a specificity of 99% at a TRAb level of 0.40 IU/L.

Conclusions: Our data showed excellent analytical and clinical performances for this novel fully automated assay with an improved specificity for stimulating antibodies.

Diagnostic and clinical significance of specific antibodies and biochemical parameters in the primary biliary cirrhosis. Study of patients from Poland

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Primary biliary cirrhosis (PBC) is a progressive, cholestatic, autoimmune liver disease characterized by changes in blood biochemical parameters and the presence of specific antibodies. The aim of this study was to present the immunological and biochemical signs of PBC and their significance for diagnosis in a well characterized cohort of patients.

Methods: We studied 160 PBC patients, 60 pathological controls with primary sclerosing cholangitis and autoimmune hepatitis, 30 healthy blood donors. Serum antimitochondrial (AMA) and antinuclear antibodies (ANA) were detected by commercial kits or by ELISA “in-house” tests.

Results: AMA were detected in 145 (91%) of PBC patients. ANAs directed against promyelocytic leukemia protein nuclear body components and nuclear envelope proteins were found in 51 (32%) and 42 (26%) cases, respectively, with specificity 97-99%. In patients sera we observed higher concentration of alkaline phosphatase – 567.2 IU/l vs 136 IU/l, γ-glutamyltranspeptidase – 335.5 IU/l vs 55 IU/l and bilirubin – 2.4 mg/dl vs 1.1 mg/dl.

Conclusions: The laboratory diagnosis of PBC base on the presence of elevated serum alkaline phosphatase, bilirubin and highly specific AMAs. Different antinuclear antibodies, frequently coexisting together, also supports the autoimmune disease, suggesting an autoimmune reaction against multiple nuclear components in some patients. The ability to detect them expands the diagnostic armamentarium of PBC-specific markers, especially in cases in which AMA are not detectable. Among ANA, anti-nucleoparin p62 antibodies have been indicated as significant prognostic markers. Their specificity for PBC was 99%. We found the association of presence of these autoantibodies with higher concentration of bilirubin.
WORKSHOPS

WS8: Roche Diagnostics

The role of Personalized Healthcare in present-day medicine

Zbigniew Gaciong
Department of Internal Medicine, Hypertension and Angiology, Warsaw Medical University

No abstract available

WS9: HORIBA Medical

HORIBA Medical introduces HELO, the new global solution for the IVD in hematology

Thomas Tran, Bruce Davis

No abstract available

WS10: Abbott Diagnostics

Laboratory medicine and Abbott solution in this field

Nora Nikolac
PhD, specialist in analytical toxicology; Head of Medical Biochemistry and Analytical Toxicology Unit at University Department of Clinical Chemistry, Medical School University Hospital Sestre Milosrdnice, Zagreb, Croatia

Marijana Miler
Specialist in laboratory medicine; Deputy head of Medical Biochemistry and Analytical Toxicology Unit at University Department of Clinical Chemistry, Medical School University Hospital Sestre Milosrdnice, Zagreb, Croatia

No abstracts available

WS11: Sarstedt AG

Preanalytical phase: Challenges convert to solutions

Christa Seipelt
Product Manager, Diagnostic & Medical Products

No abstract available

Plenary III

Promoting clinical and laboratory interaction by harmonization

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Harmonization in laboratory testing is more than merely analytical harmonization. Although the focus was mainly on the standardization and harmonization of measurement procedures and their results, the scope of harmonization goes beyond method and analytical results. It
includes all aspects of the total examination process from the “pre-pre-analytical” phase through analysis to the “post-post-analytical” phase. Rapidly available and precise results can indeed be of very limited value if they cannot be compared with each other, are produced on a wrong material or within an inappropriate diagnostic workflow. In particular, as evidence collected in last decades demonstrates that pre-pre- and post-post-analytical steps are more vulnerable to errors, harmonization initiatives should be performed to improve procedures and processes at the laboratory–clinical interface. Managing upstream demand, down-stream interpretation of laboratory results, and subsequent appropriate action through close relationships between laboratorians and clinicians remains a crucial issue of the laboratory testing process. Therefore, initiatives to improve test demand management from one hand and to harmonize procedures to improve physicians’ acknowledgment of laboratory data and their interpretation from the other hand are needed in order to assure quality and safety in the total examination process. A harmonized context will increase the value of laboratory results facilitating their interpretation and thus improving the patient’s outcome.

Plenary IV

Microbiome and disease

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Humans exist as meta-organisms comprised of both the macroscopic host and its symbiotic commensal microbiota. With approximately 100 trillion cells, bacteria outnumber host cells by at least a factor of 10 and express at least 100-fold more unique genes than their host’s genome. The tremendous enzymatic capability of the microbiome results in a plethora of metabolites found in humans which play a fundamental role in nearly all aspects of host physiology and disease development including metabolic, cardiovascular and even neuro-psychiatric illnesses.

Since 2000, large-scale 16S rRNA or metagenomic studies have dramatically expanded the knowledge about diversity of the human gut microbiome. Approximately 80% of the bacteria found by molecular tools are uncultured so far, and hence can be characterized only by metagenomic studies. On the other hand, however, culture of bacteria (microbial culturomics) increased by up to 30% the microbial gut repertoire as determined by pyrosequencing, so that distinct microbiological knowledge is clearly required to analyze the human microbiome. We completed the first European external quality assessment (EQAS) comparing results from different next generation sequencing (NGS) centers with special emphasis on critical preanalytic steps, nucleic acid preparation and bioinformatic data processing.

Furthermore, our goal is to achieve a functional understanding of bidirectional microbe-host interactions in health and diseases, beyond largely descriptive compositional and metagenomic analyses.

Session 10: Detecting age-related changes

Are age- and gender-adjusted reference values needed in aging?

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Attending physicians depend on the efficacy and effectiveness of clinical laboratories in their diagnostic and follow-up endeavours. In order to provide reliable information, laboratories need to provide meaningful reference values based on adapted stratifications of healthy people. Geriatrics is particularly sensitive to this problem as the aging process is imposing significant changes in the physiology of the individuals. Beyond biological considerations, the evolution of the technology over the last 3 decades, both at the analytical and data handling levels, calls for the redefinition of reference values.

As the aging population faces a higher incidence of morbidity compared to mid-age adults describing what is a healthy reference aging population for defining reference intervals (RIs) is challenging. The musculoskeletal system will serve as example for illustrating the need of developing age-related biomarker RIs. Aging is associated with a reduction in muscle mass and force that in turn will impact on skeletal strength through reduced mechanical load. These physiological events, independent of gender, lead to modification in bone turnover rates that could be monitored by circulating age- and gender-adjusted bone resorption and formation biomarkers, providing they are available or in development.

Aging and Oxidative Stress: Events and Methodologies in Oxidative Stress Research

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A historical account of the ‘free radical theory of aging’ will be presented, focusing on its devolvement as one of the major theories in aging research which can be tested scientifically and demonstrating findings and evidence in support of the theory. Definitions and categories in the research field of oxidative stress and antioxidants will be defined, and the concept of the ‘antioxidant redox chain’ will be elaborated. The deleterious role of reactive oxygen species (ROS) and reactive nitrogen species (RNS) in various biological systems and the damage they promote to macromolecules, will be discussed. Furthermore, common methodologies in oxidative stress research will be presented, including laboratory measurements of oxidative stress and oxidative damage to macromolecules (i.e., proteins, lipids and DNA), and various techniques for measuring antioxidant activities and/or antioxidant levels in cells and tissues and their biological implications. Finally, future perspectives in the field of oxidative stress and antioxidant research will be discussed.

Post-translational modifications derived products (PTMDPs): markers of tissue ageing?

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Laboratory of Medical Biochemistry and Molecular Biology, UMR CNRS / URCA n° 7369, Faculty of Medicine, Reims, France

Nonenzymatic post-translational modifications (NEPTMs) are chemical, non-regulated reactions which occur in human organism during all lifespan. They are responsible for protein molecular ageing, inducing alterations of their structure and functions, especially disturbing their interactions with cells. NEPTMs generate a number of complex post-translational modifications derived products (PTMDPs) which exert deleterious effects inside tissues and are involved in pathophysiological complications of ageing and various diseases such as diabetes mellitus and chronic kidney disease (CKD). The most famous NEPTM is glycation which is characterized by the binding of glucose and sugar by-products to proteins, leading to complex compounds named advanced glycation end-products (AGEs) or Maillard products. AGEs alter protein organization and react with membrane receptors like RAGE, triggering intracellular pathways leading to an enhanced oxidative stress. Another recently described modification is carbamylation, which corresponds to the binding of isocyanic acid, a by-product of urea, to protein amino groups. It generates various carbamylation-derived products (CDPs), the most abundant being homocitrulline, derived from lysine residues. Carbamylation is increased in CKD and is correlated to morbidity and mortality in patients with cardiac and renal disorders. PTMDPs accumulate in tissues during ageing and in diseases. We have recently demonstrated that carbamylation was a characteristic hallmark of ageing in three mammalian species. PTMDPs are emerging biomarkers in laboratory medicine. Their clinical significance for predicting age-related disabilities and long-term complications of diseases are currently under investigation. Non-invasive methods such as measurements of skin autofluorescence constitute also promising approaches for better understanding the role of PTMDPs in human pathophysiology.

Short Communication

Pediatric reference intervals for 1,25-dihydroxyvitamin D in the CALIPER cohort of healthy children and adolescents

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Objectives: 1,25 dihydroxyvitamin D (1,25(OH)2D) is the most biologically active metabolite of vitamin D. 1,25(OH)2D is essential to childhood growth and development and plays a role in calcium homeostasis and bone growth. Despite its importance, no pediatric reference interval exists. Traditional 1,25(OH)2D assays require complex manual preparation, however Diasorin developed a new, fully automated in vitro chemiluminescent immunoassay (CLIA), requiring no sample pretreatment or preparation. In alignment with the CALIPER(Canadian Laboratory Initiative for Pediatric Reference Intervals) project, we aimed to establish age- and sex-specific reference intervals.

Design and Methods: Blood samples were collected from healthy subjects aged 0-<19 years (n=405). Those aged 0-<1 year and 1-<19 years were Mount Sinai Hospital outpatients and CALIPER samples, respectively. 1,25(OH)2D levels were measured using Diasorin Liaison XL. In accordance with CLSI C28-A3 guidelines, R software was used to calculate age- and sex-specific reference intervals with corresponding 90% confidence intervals.

Results: There was a significant age-dependent decline in 1,25(OH)2D levels over the first few years of life requiring data partitioning and calculation of reference values for three age groups: 0-<1 year (77 - 432 pmol/L), 1-<3 years (113 - 363 pmol/L), and 3-<19 years (108 - 246 pmol/L). Sub-analysis did not suggest a seasonal effect on 1,25(OH)2D levels in our study group (p=0.364 based on the Mann Whitney U-Test) and sex-partitioning was not necessary.

Conclusions: This study provides, for the first time, robust pediatric reference intervals for the 1,25(OH)2D Diasorin Liaison assay and will improve the accuracy of pediatric test result interpretation for this active form of vitamin D.
Session 11: Clinical applications of genome sequencing

Next Generation Sequencing in undiagnosed diseases

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Undiagnosed diseases may serve as a model for the clinical application of emerging genomic technologies. State-of-the-art NGS technology is an optimal strategy to address an unmet need of clinical medicine by attaining a diagnosis for patients with complex, multisystem disorders in whom a disease is not identified despite exhaustive effort. The optimal approach is to combine NGS with dense SNP arrays which detect CNVs and pinpoint regions of homozygosity. The results need to be filtered using databases of population frequency such as 1000 Genomes database. The analysis further benefits from inclusion of data from family members to filter variants which do not segregate in a manner consistent with a Mendelian inheritance. Finally, the findings should be validated by functional studies and, preferably, by demonstrating the disease association in independently ascertained patients. The described NGS based approach has already been proven successful in a number of cases and recently National Institutes of Health (NIH) launched a dedicated the Undiagnosed Diseases Program to identify candidate genes and establish diagnosis in unresolved cases or to define new disorders. Combining extensive clinical workup with NGS is essential to reach an accurate diagnosis in unresolved cases and to discover new diseases. Enhanced collaboration of basic researchers and clinicians provides a rare opportunity to integrate this research tools into clinical practice. Rare diseases, in addition to being a diagnostic dilemma, also serve as a rich resource of the knowledge about mysterious disease mechanisms.

The use of whole exome sequencing: Clinical cases

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In 2012 Department of Medical Genetics (Warsaw Medical University) has acquired Illumina HiSeq 1500 which allowed to establish whole exome sequencing (WES) as method for both research and diagnostic purposes. Since then we have performed > 1000 WES analyses, most of which aimed at finding diagnosis in patients suspected to suffer from rare neurological disorders with a genetic basis. We also established bioinformatics infrastructure and a pipeline which allows efficient analysis of the WES data. During the lecture selected findings will be presented illustrating clinical utility of WES with emphasis on discovery of mutations associated with novel phenotypes (AIFM1 mutation in spondyloepimetaphyseal dysplasia with neurodegeneration, KIF5A mutation in myoclonic seizures and neonatal onset progressive leukencephalopathy) as well as discovery of novel diseases, i.e. those caused by mutations in genes not yet associated with known human diseases (VAC14 associated pediatric neurodegeneration).

Circulating free DNA assessment to recognize novel prognostic biomarkers in prostate cancer

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Several studies have shown the potential role of cfDNA levels in the prognostic assessment of different solid malignancies. However, the quantification of pure cfDNA is a prerequisite for a reliable genotype analysis focused on the detection of cancer-specific DNA mutations signatures and/or epigenetic modifications. In this study, the quality and quantity of cfDNA were assessed by two different quantification procedures, furthermore cancer-specific DNA mutations as prognostic biomarkers in prostate cancer patients were tested. A total of 25 prostate cancer patients and 30 aged matched healthy controls were enrolled into the study. Blood samples were collected at the diagnosis of prostate cancer, and at 6 and 12 months following the radical prostatectomy operation. Qubit 2.0 was utilized for measurements of total amount cfDNA before qPCR quantification performed targeting of the single copy gene APP. Methylated GSTP1 and RASSF1A tumour specific cfDNA markers were determined. Preliminary data showed that patients with high cfDNA concentration at baseline had worse disease free time and overall survival. The automated cfDNA extraction associated to the quantification by Qubit 2.0 seems to be the best approach to quantify the patient’s cancer-specific DNA mutations by qPCR assay. The combination of multiple mutational/methylation cancer biomarkers is suitable to determine the total amount of cfDNA in prostate cancer patients. cfDNA detection can be used as a prognostic and predictive tool for stratification, clinical management and follow-up of prostate cancer patients.
Short Communications

RHD positive variants in Moroccan blood donors serologically D negative: identification of 6 novel intronic mutations

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Background: Blood group genotyping is increasingly utilized in transfusion and obstetric medicine. In Caucasians, the presence of antigen D is predicted, if two or more RHD specific polymorphism are detected. In the presence of non-functional RHD, this prediction must fail giving false positive results. Excluding RHD pseudogene (RHDψ) and CdeS frequent in individuals of African origin, most of these variants have not been determined in Morocco.

Methods: Among a total of 544 D negative blood donors, 65 blood samples RhC and/or RhE positive were tested by serologic tests. Samples serologically D negative were screened by PCR using sequence specific priming, multiplex PCR and next generation sequencing. For novel mutations, the Alamut software was used to predict their possible effect on splicing.

Results: Seventeen samples (26.1%) previously documented as RhD negative had been missed by routine serology. The complete deletion of the RHD gene is observed in 81.2% of blood donors serologically D negative C and/or E positive. Sequencing reveals 9/48 RHD positive samples. Five new variants were detected, each one were caused by more than 2 intronic mutations. Overall, 6 novel mutations were characterized far from conserved splice sites. The bioinformatics results suggest that 3 mutations affect splicing. Two samples were CdeS allele and 2 variants were not confirmed.

Conclusion: This study is the first to describe RHD variants caused by intronic variations far from the splice sites. Further investigations in RHD gene in Maghrebian population will allow improving RHD genotyping strategy.

Pharmacogenetic studies of propofol using HPLC and long-range PCR based next generation sequencing

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Introduction: Propofol, one of the safest and commonly used anaesthetic for general anaesthesia, present a large interindividual variability in clinical response. Differences in pharmacokinetics of propofol determine the required dose of anaesthetic and the awakening time from anaesthesia. Responsible for this variability is genetic polymorphism in genes involved in action and biotransformation of propofol. Aim of our study was to identify this genetic factors.

Materials & Methods: 86 Polish patients undergoing general anaesthesia with propofol were enrolled in this study. PK of propofol was determined as mean retention time (MRT) based on HPLC measured plasma anaesthetic concentration at 5 time points after the end of infusion. We performed next generation sequencing on MiSeq of 27 DNA amplicons obtained for all subjects (in total 226,05 kbp), covering promoter (3 kbp) and coding sequence of 9 genes involved in transport (ALB, ABCB1), action (GABRA1, ADRA1A) and biotransformation (UGT1A9, CYP2B6, CYP2C9, NQO1, SULT1A1) of propofol.

Results: We observed a large interindividual variability in PK of propofol (MRT range was 10-503 min). Using NGS we determined in total 1511 variants, from which we selected 37 mutations for further analysis. Correlation test indicated, that two mutations: c.516G>T in CYP2B6 and c.2677T>G in ABCB1 gene are associated with the PK of propofol. Genotype c.516T/T determined a rapid metabolism of propofol and genotype c.2677T/G was significantly often present in the group of intermediate and poor metabolizers.

I would like to thank POLBIOGEN Foundation for financing.
Session 12: Point-of-care testing: Methodology and quality

Should performance specifications be different for POC instruments?

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POCT is the most rapidly growing field in laboratory medicine. With increasing technological and analytical possibilities, an increasing number of analyses can now be carried out on POC instruments. Although the costs of POC instruments are less than hospital instruments, the number of users of POC instruments are much larger, ranging from wards in the hospitals, GP offices, nursing homes, pharmacies and last but not least tests for self-measurements. With the increasing emphasis on patient empowerment, this is a wanted development.

The ultimate goal of using POC testing is that patient outcomes should be improved and/or that it should be more cost/effective than the use of conventional laboratory testing. To achieve this, the role of POC in the different clinical settings as well as the responsibility for introducing and manage the instruments and use of the instruments should be clearly defined. The main reason for using a POC instrument is that a rapid result is more useful than waiting for a result from a central laboratory. An essential question is therefore: Should performance specifications for POC instruments be different from that of instruments in a central laboratory. Many will say “yes”, but taking into account the different use of such instruments, performance specifications could probably be modified. Many POC instruments are used for specific clinical settings and one should therefore try to develop performance specifications for that setting. It is also probable that time and location is an important quality factor and that performance specifications can be less strict if a result is provided rapidly – especially in cases where you would like to know if the result is “very high” or “very low”; e.g. hypo- and hyperglycemia. However, if performance specifications for some POC measurement procedures should be less stringent compared to the central laboratory, it is important that this is communicated to the users of tests.

Point of Care Testing (POCT) and clinical decision making: the quality of evidence

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Point-of-care testing (POCT) has moved testing closer to the patient, with constantly evolving advances in technology and more sophisticated devices able to measure an increasing range of analytes. The major advantage of POCT is to have an instantly available result, although usually at a greater cost than routine laboratory testing and with a requirement for user accreditation, quality control, quality assurance, technical support and oversight from the clinical laboratory.

The key question is whether having a POCT result leverages clinically important decision making in a superior (or at least non-inferior) manner to having a result from the clinical laboratory. Key areas include: self-monitoring; community testing, primarily in the pharmacy; primary care; and the emergency department. In most of these areas, randomised controlled trials (RCTs) of POCT versus central laboratory testing have been undertaken, with the results of some of these trials providing support for the use of POCT. In parallel, however many of these trials have provided valuable information about how POCT should be conducted. In particular, they have shown that adoption of the technology alone is insufficient to achieve a benefit and that in many areas, the overall process is just as important.

In many countries, there is a drive to provide better and more convenient access to healthcare, particularly in subjects with chronic diseases. There is a compelling drive to adopt POCT for its perceived advantages. All cases, however need to be carefully reviewed against the evidence base and not adopted in an uncritical manner.

Molecular Diagnostics at the Point of Care

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With advancing technology, molecular diagnostics are moving into the realm of Point of Care Testing (POCT). Rapid molecular diagnostic testing is increasingly becoming important for disease identification, treatment and prevention. However, traditionally, molecular tests have been limited to specialty laboratories primarily because the technologies employed require sample purification and sophisticated instruments, are labor and time intensive, expensive, and require highly operators. With the advent of molecular POCT, with sensitivity, specificity and predictive values in the 97-99.9% range, utilizing both polymerase chain reaction (PCR) and isothermal nucleic acid methodologies, this is changing. This presentation will describe the various molecular technologies and systems currently available and discuss how they could be implemented effectively at the point of care.
Short Communication

Validation of hemoglobin and hematocrit measurements on GEM Premier 4000 blood gas analyzer

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Background: With the introduction of a new blood gas analyzer (GEM Premier 4000, Instrumentation Laboratory) in our laboratory, we preliminarily checked, in a correlation study, the interchangeability of hemoglobin (Hb) and hematocrit (Hct) results with those obtained with the automated blood cell counter used in our core-lab.

Methods: Hb and Hct were assayed in duplicate on 46 fresh EDTA whole blood samples by both the GEM analyser and the Sysmex XN-9000 hematology system. Hct was also estimated (eHct) on GEM from Hb concentrations with the formula: eHct = 0.03*(Hb/10). Correlations were performed using linear regression analysis and biases evaluated using difference plots.

Results: Hb and Hct concentrations at XN-9000 ranged from 56.0 to 175.5 g/L and from 0.16 to 0.53 L/L, respectively. Regression analysis gave the following equations: for Hb, GEM = 1.00 (95%CI: 0.97-1.03)*XN + 3.9 g/L (95%CI: 0.3-7.5), r=0.996; for Hct, GEM = 1.14 (95%CI: 1.06-1.22)*XN – 0.02 L/L (95%CI: -0.05-0.01), r=0.973; for eHct, GEM = 1.07 (95%CI: 1.01-1.13)*XN – 0.007 L/L (95%CI: -0.03-0.02), r=0.983. When compared with minimum goals for bias derived from biological variability (≤2.8% for Hb and ≤2.6% for Hct, respectively), GEM Hb displayed a slightly elevated bias (+3.4%), whereas Hct (both measured and estimated) showed a markedly positive bias (+8.7% and +4.7%, respectively).

Conclusions: GEM Hb showed a constant positive bias of ~4 g/L, which appears tolerable if the use of these results is limited to screening purposes. On the other hand, GEM Hct displayed a suboptimal accuracy, making this measurement probably unsuitable for clinical use.

Debate II: Direct oral anticoagulants (DOAC): To monitor or not?

Pro

Lotta Joutsi-Korhonen
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DOACs, thrombin inhibitor dabigatran and the FXa inhibitors rivaroxaban, apixaban and edoxaban, are already widely used for prevention of stroke in atrial fibrillation and prevention and treatment of venous thromboembolism. Clinical trials confirm that DOACs may be used effectively and safely without dose adjustment based on laboratory monitoring. Although the predictable pharmacokinetics make routine coagulation monitoring unnecessary, there are, however, circumstances in which laboratory measurements are indicated – even vital. In case of acute hemorrhage or thrombosis, emergency surgery, reversal of anticoagulation, or renal dysfunction, clinicians desire to determine the drug presence or concentration. Thus, laboratories need to have the tests readily available.

Widely available routine coagulation assays INR, PT and APTT, lack the sensitivity and are inadequate for drug monitoring. A normal APTT most likely excludes excess dabigatran, and a normal thrombin time (TT) excludes clinically relevant levels. To quantify, the dilute-TT and ecarin-based assays suit for dabigatran and chromogenic anti-FXa assay for FXa inhibitors, when calibrated with drug-specific standards.

The limitations of coagulation testing must be noted. Initially, most data have been based on drug-spiked plasma studies, rather than samples from real-life patients on DOAC therapy in clinical settings. Inter-individual variation is obvious, and coagulation methods are reagent- and laboratory-dependent. Thus, available data must be interpreted with caution and more is needed in order to establish alert values for clinicians. Laboratory could also guide the economical use of new reversal agents. These new settings and monitoring needs of anticoagulation treatment challenge clinical laboratories.

Contra:

Grzegorz Grzesk
Department of Pharmacology and Therapy, Collegium Medicum, Bydgoszcz, Poland, Nicolaus Copernicus University

No abstract available
WORKSHOPS

WS 12: Illumina

Illumina next generation sequencing oncology portfolio and vision for the future

Ilja Stap
Sr. Market Development Manager, Clinical Oncology EMEA, Illumina Inc.

No abstract available

WS 13: Becton Dickinson

Plasma: a Tool for Laboratory Testing to Help Improve Patient Management

Ana-Maria Simundic¹ Astrid Petersmann² Stephen Church³
¹ University Hospital “Sveti Duh”, Zagreb, Croatia
² University of Greifswald, Germany
³ BD Life Sciences, Oxford, UK

A fast and accurate diagnosis is recognized as a key requirement for effective patient management. Most diagnostic protocols require laboratory testing to inform or confirm the diagnosis. Recently the UK Royal College of Pathologists have recommended that time between blood collection and providing a test result, known as the ‘turnaround time’ (TAT), should be less than one hour for emergency testing. However, despite the many advances in laboratory medicine, TAT often does not meet clinician needs, potentially resulting in delays in patient treatment and management.

Serum is the predominant sample type used in medical laboratories across Europe. Serum samples must be clotted, which can take in excess of 30 minutes before processing, resulting in TAT expectations often not being met. The use of lithium heparin-based blood collection tubes to create a plasma sample eliminates clotting time and reducing TAT by 30+ minutes. The use of plasma can also lead to improvements in the accuracy of key analytes such as potassium, by reducing the potential for pseudohypokalemia. A new non-gel technology enables further improvements in TAT by reducing centrifugation time from 10 to 3 minutes, creating high quality plasma with an inert separation barrier that is suitable for a broad range of analytes. Previous sample separation technologies, such as gel, may compromise the sample by impacting the concentration of some hydrophobic therapeutic drugs over time. This new non-gel technology therefore reduces TAT and ensures the highest quality of sample in order improve patient management.

SATURDAY, September 24th

PARALLEL SESSIONS

Session 13: Pediatric endocrine symposium
Under the auspices of Asian Pacific Federation of Clinical Biochemistry

Clinical utility of steroid analysis

Tze Ping Loh
Department of Laboratory Medicine, National University Hospital, Singapore

Steroid hormones are lipid-soluble hormones that are produced mainly in the adrenal cortex (corticosteroids) and gonads (sex hormones, also produced in the placenta). They exert a wide variety of effects, influencing the stress response, immune function, inflammation, metabolism, electrolyte and water homeostasis, and sexual development of a person. Steroid hormones are of vital importance to the paediatric population, who are undergoing continuous grow and development. Any disturbance to the normal steroid hormone function in childhood may lead to significant morbidity and even mortality. On the other hand, the clinical impact of steroid hormone defect may be modified by the stage
of development of a child. In the paediatric population, steroid hormones are often measured in suspected cases of adrenal disorders (congenital adrenal hyperplasia, Cushing’s syndrome, disorders of sexual differentiation, aldosterone problems), delayed/precocious puberty and hirsutism. The diagnosis of steroid hormone disorder is challenging in the paediatric population, as they often are unable to/ do not complains of the clinical symptoms related to hormone dysfunction and the clinical signs can be mild and variable. Yet, it is essential that early and accurate diagnosis be made to ensure the child grows up healthily. Hence, accurate analysis of steroid hormone in this population is particularly important. In this overview lecture, we will briefly review the physiology of steroid hormones in relation to the growth and development of a child. Following this, through a few case examples, we will explore how defects in the certain steroid hormone pathways may result in clinical disease, and illustrate the importance of steroid hormone analysis in the management of such patients. Finally, we will explore the concept of vitamin D as a steroid hormone and its implication for health in the paediatric population, beyond its effect on calcium metabolism and bone health.

**Routine steroid hormones service by mass spectrometry for pediatric endocrinology**

**Chung Shun Ho**  
*Bmioc Medical Mass Spectrometry Unit, Department of Chemical Pathology, The Chinese University of Hong Kong, Hong Kong*

Routine service for the measurement of steroid hormones is important for the diagnosis and monitoring of pediatric patients suffering from endocrine disorders. Automated immunoassay platforms are commonly used and the methods are sensitive to meet clinical needs. Unfortunately immunoassays are notorious in lack of specificity due to cross-reactions with metabolites and other steroids with similar molecular structures. Mass spectrometric methods are sensitive and specific. Gas chromatography-mass spectrometry (GCMS) methods have been developed as reference methods for the quantitation of steroid hormones in biological samples. However, GCMS methods are labor intensive and time consumption, usually require derivatization of the steroid hormones prior to mass spectrometric analysis, rendering it to be less favorable for routine service in the clinical laboratories.

In our laboratory, liquid chromatography electrospray ionization tandem mass spectrometry (LCMS/MS) methods have been developed to measure steroid hormones for routine laboratory service. Sample preparation procedures are simple and the methods are robust. Analytical performance of these methods is comparable with methods published in the literature and they are fit for clinical purposes. Weekly / biweekly routine services are available for 17-hydroxyprogesterone, 25-hydroxyvitamin D2 /D3, aldosterone, androstenedione, cortisol, dihydrotestosterone and testosterone in serum / urine samples. In this presentation, technical challenges in developing and maintaining these routine methods are discussed, such as sample preparation, calibrator matrices, stable isotope labeled internal standards, ionization modes, external quality assurance and staffing requirement. Our experience concludes that measurement of steroid hormones by LCMS/MS is feasible for routine service in a clinical laboratory.

**Interpreting mass spectrometry data for the diagnosis of disorders of sexual development**

**Stefan A. Wudy**  
*Steroid Research & Mass Spectrometry Unit, Laboratory for Translational Hormone Analytics, Pediatric Endocrinology & Diabetology, Center for Child and Adolescent Medicine, Justus Liebig University, Giessen, Germany*

Since the earliest identification of steroids by mass spectrometry (MS) in the sixties of last century, rapid technical progress has enabled development of clinically applicable techniques for determining steroids based on MS. Gas chromatography (GC) has the highest separation power for steroids and in combination with MS (GC-MS or GC-MS/MS) enables targeted as well as non targeted steroid analysis. Electron impact ionization yields a high degree of structural information and thus enables highest specificity. In case of MS/MS an extra level of filtering is provided. Since the late eighties of last century, liquid chromatography (LC)-MS/(MS) has come of age. In this technique, soft ionization leads to simple spectra with hardly any fragmentation. The technique is mostly used in a targeted approach. It proves particularly suitable for the analysis of the intact steroid, e.g. in case of complex steroids. Furthermore simple sample work up and short run times allow for high throughout analysis.

This lecture will deal with the application of the above-mentioned mass spectrometric techniques in the differential diagnosis of steroid related disorders, particularly disorders of sexual development. This will be demonstrated by typical examples from urinary and plasma steroid analysis with typical steroid profiles and metabolomics approaches. Furthermore it will be shown that GC-MS and LC-MS are not competing but complementary analytical techniques.

**Mass spectrometry reference intervals for serum steroids**

**Ronda F. Greaves**  
*School of Health and Biomedical Sciences, RMIT University, Victoria, Australia  
Centre for Hormone Research, Murdoch Children’s Research Institute, Victoria, Australia  
APFCB Scientific Committee*
Session 14: Biomarkers in neurology

Novel biomarkers for the early detection and tracking of Alzheimer’s disease

Michael Ewers
Institute for Stroke and Dementia Research (ISD), Ludwig-Maximilians-University Munich

In Alzheimer’s disease (AD), pathological brain changes start to emerge years before the onset of dementia symptoms. The assessment of disease-specific brain changes may enable the early detection of AD and thus provide a time-window for secondary intervention in AD. We have reported that structural MRI shows a characteristic pattern of accelerated atrophy in temporal and parietal brain regions in cognitively normal subjects with amyloid pathology. Multivariate statistical analyses showed that such patterns are predictive of accelerated rates of memory decline at this early stage of AD. We developed novel machine learning algorithms applied to structural MRI that allow for the detection of elderly cognitively normal subjects with emerging amyloid pathology. These results suggest that neuroimaging based biomarkers are a useful tool to identify pre-symptomatic subjects at increased risk of AD. An important factor that modulates the association between brain pathology and cognitive changes in AD is cognitive reserve. Elderly subjects with higher cognitive reserve can maintain cognitive function at higher levels of AD pathology. Based on resting-state functional MRI (fMRI), we found that increased neural network integrity, predominantly within the frontal lobe, was associated with relatively stable cognitive function despite the presence of early AD pathology. We developed an fMRI based biomarker of increased frontal connectivity that allowed for the prediction of higher cognitive reserve capacity. Such fMRI markers of reserve may allow to improve prediction models of the occurrence of dementia symptoms in elderly subjects with emerging AD pathology.

Another important modulating factor in AD is the neuroimmune response to emerging amyloid pathology. Rare mutations in the gene which encodes the TREM2 receptor expression on microglia are associated with increased risk of AD dementia. We have shown that changes in the levels of the protein TREM2 in the cerebrospinal fluid (CSF) are dramatically increased in non-demented elderly subjects with increased amyloid and tau pathology. These results were observed in AD cases without TREM2 mutations, suggesting that TREM2 related neuroimmune-changes play a wider role in the early phase of AD. Based on findings in subjects with autosomal dominant AD, I will discuss the role of TREM2 in grey matter atrophy in presymptomatic subjects. In conclusion, multivariate statistical approaches applied to multi-modal imaging of structural and functional grey matter changes trace the evolution of AD and allow for the prediction of cognitive decline at the pre-dementia stage. Such predictive biomarker models need to be informed by modulating factors such as functional brain changes of cognitive reserve and neuroinflammation.

Early and late biochemical events in neurodegeneration

Andrzej Szutowicz
Department of Laboratory Medicine, Medical University of Gdańsk, Poland

The loss of neurons and suppression of energy metabolism, in pathology-affected areas of the brain, are characteristic features of several neurodegenerative conditions including Alzheimer’s disease, and vascular, dialysis, alcohol, liver, or thiamine deficiency encephalopathies. Several acute neurotoxic insults including transient hypoxia, hypoglycemia, or xenobiotics may generate excess of free radicals, glutamate-Zn excitotoxic stimulation, trace metal dis-homeostasis hyper-ammonemia and others, may pave the path for subsequent stages of neurodegeneration. Preferential impairment of central cholinergic neurons is blamed for appearance of cognitive deficits leading to dementia in final stages of these pathologies. This phenomenon may result from the fact that cholinergic, unlike other neurons utilize a direct key energy precursor metabolite - acetyl-CoA, derived from glucose, not only for ATP and N-acetylaspartate synthesis but also for acetylcholine production. Such conditions promote amyloidogenic processing of amyloid-β precursor protein, yielding accumulation of neurotoxic amyloid-β oligomers, which may further aggravate primary neurotoxic signals through intracellular and extracellular interactions. Pathological hyper-phosphorylation of microtubule-associated tau protein, results in its intraneuronal aggregation. P-tau released from disintegrated neurons accumulates in extracellular compartment of the brain. This presentation basing on cellular, animal models and clinical-laboratory medicine data, describes putative mechanisms linking early pathological alterations in energy-acetyl-CoA metabolism with late stages of different cholinergic encephalopathies.
Brain in the fire: Laboratory diagnosis of brain autoimmune diseases

Krystyna Szymanska1, Anetta Jeziorek2, Elżbieta Stawicka2, Iwona Sawionek2, Urszula Demkow3

1 Department of Child Psychiatry, Medical University of Warsaw
2 Neurodiagnostic team, SPDSK, Warsaw
3 Department of Laboratory Diagnostics and Clinical Immunology of Developmental Age, Medical University of Warsaw, Poland

Autoimmune diseases of nervous system can be secondary to cancer or infections or idiopathic and can affect all structures of the nervous system including brain, cerebellum, cranial nerves, spinal cord and peripheral nervous system.

The authors present difficulties in the diagnosis on the example of five patients with a variety of autoimmune central nervous system diseases. The diagnosis was based on clinical picture, neural autoantibody detection and improvement after initiation of immunotherapy. All patients had autoantibodies against various structures of CNS: NMDAR, GAD, MA2/Ta and anti-sulfatide.

Short communications

Kappa free light chain as a diagnostic tool in multiple sclerosis

Bruna Andreguetto1, Paula Bottini2, Celia Garlipp1, Marco Moda2, Maria Ines Souza2

1 Department of Clinical Pathology, University of Campinas, Brazil
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Multiple sclerosis (MS) is a chronic demyelinating disease of the central nervous system that affects mostly young adults. Oligoclonal IgG bands (OCB) in cerebrospinal fluid (CSF) is observed in up to 90% of patients with MS, being isoelectric focusing electrophoresis (IEF) considered the gold standard for its detection. This procedure is time-consuming and prone to interoperator variability. Recent studies have suggested that increased levels of kappa free light chains (KFLC) in CSF may support MS diagnosis. The aim of this study is to evaluate the performance of KFLC as a diagnostic tool in MS.

KFLC (nephelometry, N Latex FLC Kappa, BNII-Siemens) and OCB (IEF, SPIFE® IgG IEF-Helena Laboratories) were analyzed in 141 paired CSF/serum samples. Paired t-test and kappa correlation were used for statistical analysis.

Both KFLC concentration in CSF and KFLC CSF/serum ratio were significantly higher in patients with OCB (p < 0.001). The cutoff value of 0.90 mg/L for KFLC showed high negative predictive value (89%) and specificity (94%) in identifying patients with OCB (r = 0.9000, kappa = 0.718, p < 0.001). Ninety-five patients had neurological disorders other than MS. In this group, 82 patients had OCB and KFLC negatives while 6 of them showed OCB and KFLC positives. Forty-six patients had MS or possible MS. Among these patients we observed OCB+ /KFLC- (n = 29); OCB+/KFLC- (n = 7); OCB-/KFLC- (n = 7) and OCB-/KFLC+ (n = 3). KFLC in serum did not differ between groups, suggesting intrathecal production.

Our data shows that KFLC determination can safely exclude patients without MS, besides being an automated, quantitative and easy to standardize assay.

The usefulness of the assessment of the cerebrospinal fluid YKL-40 and VILIP-1 concentrations in Alzheimer’s disease

Pawel Muszynski1, Agnieszka Kulczynska-Przybik1, Aleksandra Klinkowicz-Mrowiec1, Agnieszka Slowik1, Piotr Lewczuk1, Maciej Smitkowski4, Barbara Mroczko1

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4 Department of Biochemical Diagnostics, Medical University of Białystok, Poland

Background: Chitinase-3-like protein 1 (YKL-40) - a putative inflammatory marker, and visinin-like protein-1 (VILIP-1) - a neuronal injury marker, could be useful for detection of pathophysiological processes in Alzheimer’s Disease (AD).

Aim: The purpose of this study was to examine utility of cerebrospinal fluid (CSF) proteins, YKL-40 and VILIP-1, as diagnostic and prognostic biomarkers in AD, and to compare them to the CSF levels of biomarkers used in neurochemical dementia diagnostics (NDD). Study patients were carefully selected using Erlangen score and other biochemical markers, e.g. CSF/serum albumin and CSF/serum immunoglobulin quotients.

Methods: YKL-40, VILIP-1, Aβ-40, Aβ-42, hTau and pTau were analyzed in CSF of 21 AD patients and 9 elderly subjects without cognitive decline using ELISA. CSF and serum concentrations of albumin and immunoglobulins were measured with nephelometry.

Results: Expectedly, NDD biomarkers differed significantly between the groups. The CSF concentrations of VILIP-1 and YKL-40 were significantly higher in the AD patients compared to the controls. CSF concentrations of YKL-40 and VILIP-1 correlated negatively with Aβ42/40 ratio. Additionally, elevated CSF levels of YKL-40 and VILIP-1 correlated positively with increased concentrations of CSF hTau and pTau.
Conclusion: Our results suggest that VILIP-1 and YKL-40, in combination with classical NDD biomarkers, may improve diagnosis of patients with AD. Further studies are needed on larger patient groups.

Acknowledgement: The study was conducted with the use of equipment purchased by Medical University of Białystok as part of the RPOWP 2007-2013 funding, Priority I, Axis 1.1, contract No. UDA-RPPD.01.01.00-20-001/15-00 dated 26.06.2015.

Session 15: New trends in Allergy testing

Food and inhalant allergy

Randolf Brehler
University Hospital Muenster, Department of Dermatology

The prevalence of IgE mediated allergic diseases as allergic rhinoconjunctivitis, allergic asthma, and food allergy is increasing worldwide. The detection of antigen specific IgE-antibodies is pivotal in order to provide the diagnosis of sensitisation in affected individuals. Natural allergen extracts are complex mixtures consisting of major and minor allergens, they contain also non-allergenic material. The component resolved diagnostics are a new approach offering the option to detect sensitisation to a single allergen contained in such a complex mixture. Some allergens are highly characteristic and specific for an allergen source and sensitisation against such an allergen demonstrates genuine sensitisation against this source. Other proteins are pan-allergens and homologous proteins can be found in allergen extracts of unrelated allergen sources. Sensitisation against such an allergen is therefore the course of a positive result of specific IgE determination against several extracts of biologically unrelated allergen sources.

Many allergens are glycoproteins and specific IgE-antibodies can be directed to protein or carbohydrate determinants. IgE-antibodies against carbohydrate determinants are an important cause for serologically cross-reactivity to various allergen extracts and are normally not of clinical importance. Recombinant proteins expressed in E. coli bacteria are not glycosylated and sensitisation against those recombinant proteins demonstrate therefore sensitisation against protein epitopes.

Sensitisation to some proteins is highly related with clinical relevance, and if they are associated with severe allergic reactions they are called “risk allergens”, while sensitisation to other proteins is normally of low clinical relevance “low risk allergens”.

Component resolved diagnostic offers relevant advantages for the routine allergy diagnostics:

- Allergens can be grouped in allergen families - Bet v 1 is the birch pollen major allergen representing the PR-10 allergen family, responsible for cross-reactivity with tree nuts, and stone and pomaceous fruits.
- Differentiation between crossreactivity and primary sensitisation – s.-IgE to birch pollen extract will be specific for birch pollen allergy if the patient is sensitised against the major allergen Bet v 1. If the positive serological result is related to sensitisation against the profilin Bet v 2 solely the patient will be probably primarily sensitised to another allergens source; grass pollen allergic patients are frequently sensitised to profilins. In the case of sensitisation to CCD determinants and not to protein epitopes of birch pollen allergens the patient is definitely not allergic to birch pollen.
- Identification of sensitisation to risk allergens - sensitisation to the peanut storage protein Ara h 2 is associated with a higher risk for anaphylactic reactions compared with the sensitisation to Ara h 8, a homogeneous protein to Bet v 1, associated mainly with mild allergic reactions in primary birch pollen allergic patients. In the case of sensitisation to CCD determinants and not to protein epitopes of peanut allergens the patient is definitely not allergic to peanut ingestion.
- Increased analytical sensitivity - in E. coli bacteria expressed allergens are CCD-free so that IgE-antibodies directed to the mostly clinically not relevant CCD epitopes are not detected.

Due to manufacturing procedures natural allergen extracts may contain some allergens in a very low concentration resulting in an insufficient sensitivity of the test. Analytical sensitivity of the test can be increased by the use of such a single protein or by spiking natural extracts with components.

Allergen component testing for food allergy: ready for prime time?

Urszula Demkow
Department of Laboratory Diagnostics and Clinical Immunology of Developmental Age Medical University of Warsaw, Poland

Accurate diagnosis of atopic allergy is important to select targets for immunotherapy, avoid serious allergic reactions and predicts and understand cross – reactivity among allergens. Skin prick testing and serum specific IgE levels are sensitive tests however both are based on allergen extracts. Advances in the identification of clinically-relevant allergens and the development of recombinant proteins allow for assessment IgE binding to individual epitopes. Such tests are known as component-resolved diagnosis (CRD). Increased accuracy of CRD is achieved by assessing IgE binding to separate antigenic epitopes. CRD may also provide additional prognostic information regarding the severity or persistence of allergies. CRD is available for a large panel of different allergens (food, inhalant, venoms) – as protein microarrays or immunoblot tests. CRD have the potential to provide a more accurate diagnosis of allergic reactions to a variety of food types including milk, eggs, peanut and various fruits. Clinicacl cases and recommendations for CRD will be presented.
Drug hypersensitivity

Stefan Woehrl
Floridsdorf Allergy Center (FAZ), Franz-Jonas-Platz 8/6, A-1210 Vienna, Austria

Up to 8% of the general population report about adverse drug reactions (ADRs). Unevaluated ADRs lead to the prescription of less effective or more expensive alternative drugs. Hence, a work up is recommended.

A) Type A reactions ('Augmented') are related to the pharmacological action of the drug, dose-related, somehow predictable and can appear in all patients. 80% of all ADRs are type A reactions. E.g. gastrointestinal bleeding by non steroidal anti-inflammatory drugs (NSAID), leukopenia caused by chemotherapy, high blood glucose levels and elevated blood pressure to corticosteroids, etc.;

B) Type B reactions ('Bizarre') are less frequent and compose around 20% of ADRs. They are unpredictable, not dose-related, potentially dangerous and will only appear in some pre-disposed patients; e.g. true drug allergies (such as penicillin allergy), NSAID intolerance (urticarial/angioedema/asthma), malignant hyperthermia to muscle relaxants;

The most important step in the work-up of an ADR is obtaining a detailed patient's history and classifying type A/B reaction. Causality assessment is important in type A reactions for pharmacovigilance but not on an individual base. Hence, no individual work-up is recommended for type A-reactions.

The lecture will mainly deal with the work up of type B reactions. The most frequently affected organs are skin, liver and haematological abnormalities. The most important diagnostic steps are history, skin test and provocation tests. For safety and convenience, good in vitro tests would be highly welcomed, however, they are hardly available and those that exist have a good specificity but an insufficiently low sensitivity.

WORKSHOPS

WS 14: Digital Publishing

Digital publishing in clinical chemistry and laboratory medicine

T.S. Pillay (1)(2)
(1) Department of Chemical Pathology, University of Pretoria & National Health Laboratory Service, Pretoria; (2) Division of Chemical Pathology, University of Cape Town, South Africa

The evolution of the paperless society has driven the digital publishing of academic textbooks rapidly forward with the development of software and hardware and the adoption of tablets and mobile devices in institutions. Many publishers are adopting these platforms because of the benefits: easy and rapid update of interactive content; monitoring of usage and uptake and assessment of learning within the interactive content. In addition, prices can be maintained at a low level making it more accessible across the world especially in developing countries.

The rapid proliferation has generated a number of different formats and this can be problematic for an author to choose. As a result, the International Digital Publishing Forum (IDPF) developed uniform standards for electronic publishing in the so-called ePub3 format. EPUB 3 is the latest version of EPUB and is based on the latest HTML5 standard, which allows publications to contain video, audio, and interactivity as found in modern browsers. EPUB 3 facilitates the adaptation of content display to the screens and this is one of the key characteristics that distinguishes EPUB from PDF, a portable document format designed to represent print-replica content. PDF was designed to mimic paper and is not suited to digital readers in a multi-screen world and is an isolated silo technology not aligned with HTML5 and the modern web platform. Publishers can create a single EPUB file and deliver it to all distribution channels that accept the EPUB format. I will present examples of the different delivery formats in this presentation.

WS15: Roche Diagnostics

Laboratory diagnostics for identifying individuals at risk for cardiovascular diseases events

Sanja Stankovic
Center for Medical Biochemistry, Clinical Center of Serbia, Belgrade, Serbia

No abstract available
WS16: EFLM workshop: Progress in achieving recognition of specialists in laboratory medicine

Gilbert Wieringa
Laboratory Medicine, Bolton NHS Foundation Trust, BOLTON, BL4 0JR

No abstract available

Plenary V

Clinical Application of Companion Diagnostics

Jan Trøst Jørgensen
Dx-Rx Institute, Fredensborg, Denmark

Despite the hype about precision medicine in recent years, most drug prescriptions are still largely based on ‘trial and error’ and not on solid pharmacogenomic biomarker data. Such an approach can have serious medical consequences for the individual patient as well as economic consequences for the healthcare system. For most serious chronic diseases, early diagnosis and early intervention are two elements of key importance and the intervention needs to be correct. A companion diagnostic assay is defined as an in vitro diagnostic test that provides information that is essential for the safe and effective use of a corresponding therapeutic product. These assays are most often developed in parallel to the drug, using the drug diagnostic co-development model. The success of this model depends very much on the strength of the biomarker hypothesis deduced during the early research and preclinical phases of the development of a drug. So far, companion diagnostic assays have more or less been reserved for oncology, where they have played a decisive role, which has resulted in improved efficacy for a number of drugs. However, even within oncology the number of drugs that have a companion diagnostic linked to their uses is still limited, despite the drug-diagnostic co-development model has been known for nearly 20 years. Consistent use of pharmacogenomic biomarkers would lead to a more rational and cost effective pharmacotherapy to the benefit of the individual patient as well as the healthcare system as a whole.

4th Joint EFLM-UEMS Congress

Thursday September 22nd

Abstracts selected for presentation during poster walks

Laboratory biomarkers of cardiovascular disease

Abstract number 0079

Evaluation of immature platelet fraction and SIRS diagnosis after cardiac surgery

Claudia Imperiali1, Macarena Dastis-Arias1, Juan Carlos Lopez-Delgado2, Lourdes Sanchez-Navarro3, Isabel Cachon-Suarez3, David Barbel-Francol, Dolores Dot-Bach1
1 Laboratory Medicine, Bellvitge University Hospital, Spain
2 Intensive Care Unit, Bellvitge University Hospital, Spain

Introduction: Hematological analyzer Sysmex XN (Roche Diagnostics) incorporated a fluorescent channel to measure Immature Platelet Fraction (IPF). Recent investigations suggested IPF could be an accurate inflammatory biomarker that reflects bone marrow activity.

The aim of this study was to evaluate the association of IPF with Systemic Inflammatory Response Syndrome (SIRS) in hospitalized patients at Intensive Care Unit (ICU) after cardiac surgery (CS).

Methods: Prospective study was carried out on 102 patients. IPF was measured in blood samples collected in EDTA-K3 at 0h and 24h after CS in Sysmex XN2000 analyzer (Roche Diagnostics). dIPF was defined as a difference between 24h and 0h IPF results. SIRS diagnosis was defined as the need of vasopressors >24h to maintain adequate mean arterial pressure. To evaluate dIPF and SIRS association, comparisons between both groups (SIRS/no-SIRS) were made using Wilcoxon-Test to assess the significance of the difference between groups, and logistic regression and Odds Ratios were estimated. Statistical analyses were performed using STATA®.

Results: Out of the 102 patients included, 25,5% fulfilled SIRS criteria. The mean value of dIPF in SIRS and noSIRS groups were 1,32% and 0,26%, respectively. The result of the means comparison among these groups indicated a significant difference (p=0,0135). The logistic regression showed a statistical significance association between dIPF and SIRS (p=0,002), with an Odds Ratio 1,80 [IC95%: 1,23-2,62].

Conclusions: According with our results, dIPF increase the SIRS risk after CS. Our preliminary results suggest that IPF may be a potential biomarker of SIRS in the setting of CS. However, further analysis and more consistent data are needed.
Abstract number 0164

Short-term kinetics of troponin I and galectin-3 in acute myocardial infarction

Marcello Ciaccio1, Bruna Lo Sasso1, Luisa Agnello1, Giuseppina Novo1, Giulia Bivona1, Chiara Bellia1, Concetta Scazzone1, Salvatore Milano2, Stefano Favero3, Claudio Galli3

1 Department of Biopathology and Medical Biotechnologies, Università degli Studi di Palermo, Italy
2 2Biomedical Department of Internal and Specialist Medicine, Università degli Studi di Palermo, Italy
3 Complex Operating Unit of Laboratory Medicine - CoreLab, A.O.U.P., Palermo, Italy
4 Medical Scientific Liaison Europe, Abbott Diagnostics, Italy

Study aims: Assess the levels and short-term kinetics of troponin I and Galectin-3 in myocardial infarction (MI).

Methods: MI patients from whom two samples (baseline – T0 and after 5 days -T5) were available. Samples were assayed for high sensitive troponin I (hsTnI) and Galectin-3 on the Abbott ARCHITECT analyzer. Absolute levels and quantitative differences over time were calculated overall, by gender and between MI with or without ST segment elevation (STEMI/NSTEMI).

Results: Out of 217 patients (160 males (M), 57 females (F); mean age 64.4 ± 13.7 years, female older) NSTEMI was diagnosed in 37.6% of M and in 45.3% of F. HsTnI was detectable at T0 in all (range: 2 to >50,000 ng/L) and 90.7% exceeded the 99th percentile (females: 16 ng/L; 96.4%; males: 34 ng/mL; 84.7%). Median levels were higher in NSTEMI (3,142 vs. 1,012 ng/L). At T5 mean hsTnI decreased from 22,509 ng/L to 7,316 ng/mL; a decrease was observed only in 54.5% of cases. Galectin-3 levels exceeding the 97.5th percentile were found in 23.1% at T0 (M: 20.4%; F: 30.9%; p < 0.05), more frequently in STEMI (26.2% vs. 18.1% in NSTEMI; p < 0.05). At T5 the difference from baseline ranged from -80% to +266%; in 43.1% of cases variations exceeded the proposed short-term reference change value (RCV) of 27%.

The wide range of short-term variations in hsTnI may be ascribed to the different type and extension of AMI and to treatment. The variations of Galectin-3 were unexpected: different patterns of cardiac remodeling shall be ascertained by a long-term follow-up.

Abstract number 0188

Searching for a BNP standard: glycosylated proBNP as a common calibrator enables improved comparability of BNP immunoassays results

Alexander Semenov1, Tamm Natalia1, Fred Apple2, Karen Schulz2, Sara Love2, Feygina Evgeniya1, Alexey Katrukha1

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3 School of Biology, Moscow State University, Moscow, Russian Federation

Background: Circulating B-type natriuretic peptide (BNP) is widely accepted as a useful and cost-effective biomarker of cardiac function. Clinical studies suggest that there are significant differences in measured values among different commercial BNP immunoassays. The purpose of our study was to compare BNP-related proteins to determine a form that could be used as a common calibrator to improve the comparability of commercial BNP immunoassays.

Methods: BNP was measured in forty EDTA-plasma samples from heart failure patients (both acute and chronic) using five commercial BNP assays: Alere Triage, Siemens Centaur XP, Abbott I-STAT, Beckman Access2 and ET Healthcare Pylon. Six preparations containing BNP 1-32 were used as external calibrators for each assay: synthetic BNP (Bachem), recombinant BNP (expressed in E. coli; RayBiotech), recombinant nonglycosylated proBNP (expressed in E. coli; HyTest), recombinant His-tagged (N-terminal) nonglycosylated proBNP (expressed in E. coli; RayBiotech), recombinant glycosylated proBNP (expressed in HEK cells; HyTest) and recombinant glycosylated proBNP (expressed in CHO cells; HyTest).

Results: Using the internal standards provided by manufactures and five out of six external calibrators showed up to 3.5-fold differences for BNP values. However, a marked reduction of the between-assay variability was achieved, with regression line slopes close to 1.0 for almost every pair of assays, when glycosylated proBNP (expressed in HEK cells) was used as the common calibrator for all assays.

Conclusions: These data suggest that recombinant glycosylated proBNP may serve as a common calibrator for BNP immunoassays to reduce between-assay variability and achieve better comparability of BNP values of commercial BNP immunoassays.
Abstract number 0205

The serum level of miR-362-5p and miR-10b-5p discriminate patients with dilated cardiomyopathy from healthy individuals

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Aim of the study: The potential diagnostic value of specific, circulating miRNAs in blood of patients with non-ischemic dilated cardiomyopathy (DCM). We assessed the hypothesis that the relative level of specific miRNAs in serum obtained at baseline will discriminate patients with DCM from healthy individuals.

Methods: We enrolled 10 consecutive patients with DCM who underwent transendocardial CD34+ cell transplantation between January and December 2013 (9 men, 1 woman, median age 56, range 42 to 68 years, Group A) and 6 healthy individuals (3 men, 3 women, median age 58, range 38 to 59 years, Group B). Total RNA was isolated from serum with the miRCURY RNA Isolation Biofluids Kit. cDNA synthesis and real-time qPCR were performed using the miRCURY Locked Nucleic Acid and Universal RT microRNA PCR system (all Exiqon, Denmark) with the qPCRs were run on a ViiA7 thermocycler.

Results: When analyzing the pool of miRNA (752 different miRNAs assays) we found the serum level of miR-362-5p and miR-10b-5p to be significantly different between two study groups (both had adjusted P values < 0.05). The serum levels of miR-362-5p and miR-10b-5p were lower in the group of DCM [log2 fold change of -4.138 (adjusted P value = 0.026) and log2 fold change of -1.141 (adjusted P value = 0.046), respectively].

Conclusions: The relative serum values of miR-362-5p and miR-10b-5p appear to discriminate between DCM and healthy individuals and could be used as a potential diagnostic biomarkers in this cardiovascular disease.

Abstract number 0256

Microvascular and macrovascular involvement in systemic lupus erythematosus (SLE) – preliminary data

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Aim: The study was designed to evaluate the association between cerebrovascular and cardiovascular involvement as well as nailfold capillaroscopy (NC) abnormalities and immunologic, inflammatory and classical risk factors in SLE patients.

Methods: The patient group consisted of 30 persons. Imaging studies included: bilateral transcranial doppler (TCD), MRI scans of the brain, 3D contrast-enhanced MR angiography of carotid arteries, carotid intima-media thickness (cIMT) measurement and NC.

More than 100 variables were taken into account including cytokines, inflammatory/immunological markers, classical risk factors and selected organ manifestations.

Statistical analysis: chi2 Yates, chi2 Pearson, rank Spearman correlations tests and logistic regression analysis.

Results: Factors which significantly correlated with analyzed vascular changes including microemboli in TCD, ischemic changes in MRI and NC abnormalities, were thrombocytopenia (r=0.47, p=0.01), C-reactive protein (r=0.51, p=0.0039) and antiphospholipid antibodies (aPLs) (r=0.55, p=0.0015). There was significant association between vascular endothelial growth factor (VEGF) and IL-6 and high cIMT (r=0.36, p=0.0492, r=0.41, p=0.0239, respectively). Additionally, patients with changes in NC significantly more frequently were dyslipidemic (r=0.56, p=0.0015), hypertensive (r=0.41, p=0.0252) and unveiled cardiac involvement (r=0.38, p=0.0441). There was important positive correlation between cIMT and NC abnormalities (r=0.40, p=0.0300) and microemboli in TCD (r=0.44, p=0.0211). Finally, microemboli in TCD were associated with MRI ischemic changes (r=0.45, p=0.0177).

Conclusions: NC and cIMT provide the optimal protocol to screen SLE patients for cardiovascular risk. Inflammatory markers and aPLs seem to be crucial pathogenic factors in micro- and macrovascular impairment development in SLE. Patients with higher cIMT and aPLs should undergo TCD for cerebrovascular risk assessment.
Towards standardisation of 1-32 brain natriuretic peptide measurements

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B-type Natriuretic Peptide is a 32 amino acid cardiac hormone routinely measured by immunoassays to diagnose heart failure. While it is reported that immunoassay results can vary up to 45% due to cross-reactivity of different assay architectures and lack of calibrator characterisation, no attempt of standardisation or harmonisation through the development of certified reference materials (CRM) or reference measurement procedures has yet been carried out. In order to develop a method for quantification of BNP in plasma traceable to the System of International Units (SI) i. a BNP primary standard was prepared, ii. a stabilisation protocol for BNP in plasma was optimised, iii. a two steps sample clean up procedure combined with a liquid chromatography mass spectrometry method (QQQ) was developed and validated. The application of the method to a number of samples from the UK NEQAS (National Quality Assessment Scheme) cardiac marker scheme showed that LC-MS analysis provides consistently lower results than immunoassays underlying the poor definition of the measurand of immunoassays and showing potential for standardisation.

Nephrin and podocalyxin - markers for early detection of diabetic nephropathy

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Introduction: Diabetic nephropathy is the leading cause of end-stage renal disease. Podocytopathies have crucial role in pathogenesis of diabetic nephropathy, thus the podocyte proteins have significance in early detection this disease. The purpose of this paper is to test the significance of nephrin and podocalyxin as markers for early detection of diabetic nephropathy.

Material and Methods: This study included 82 people with type 2 diabetes (36 male/46 female) with average age 56.6 ± 6.7. As control group were included 30 healthy subjects (10 male/20 female) with average age 48.7 ± 9.4. All patients were divided into three groups: normoalbuminuria, microalbuminuria and macroalbuminuria. We used fresh urine and venous blood. In urine: nephrin and podocalyxin by ELISA method, creatinine - photometrically and microalbumin – turbidimetrically were measured. In addition we measured blood urea, creatinine, glucose, albumin and total protein by photometric method.

Results: In 74.6% /52.5% of normoalbuminuric subjects with type 2 diabetes we found elevated urinary nephrin/ podocalyxin respectively. Nephrin and podocalyxin were significantly elevated in in all groups of participants with diabetes compared with the control group (p < 0.05). Both markers were significantly elevated in group of patients with normoalbuminuria compared with the control group (p < 0.05). We found positive correlation between urinary concentration of nephrin and podocalyxin and serum creatinine and negative correlation between these urinary markers and eGFR. ROC analysis showed that both markers have significant diagnostic efficacy in diabetic nephropathy.

Conclusion: Nephrin and podocalyxin can be useful markers for early and non-invasive detection of diabetic nephropathy.
Abstract number 0027

Stabilization of glucose concentration in the new VACUETTE® FC mix blood collection tube for diagnosis of gestational diabetes

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Background: Reliable detection of Gestational Diabetes Mellitus (GDM) is required to prevent maternal and fetal complications. The study shows long-term stability of initial glucose concentration in specimens spun directly after collection and compared to whole blood specimens stored at room temperature.

Method: The study was conducted at ISALA Hospital using VENOSAFE™ FC Mixture versus VACUETTE® FC Mix tubes. Forty-three pregnant donors (healthy and GDM-diagnosed) were recruited. Venous blood was drawn into four tubes (two tubes each tube type). One tube of each type was spun directly after collection and the others in whole blood after 48h at room temperature. Plasma was measured immediately after centrifugation to obtain initial values (fasting) and after 48h for evaluation of glucose stability (Hexokinase method, COBAS 8000).

Results: Evaluation of clinical results for glucose concentration was done on basis of maximal allowed deviation for a single value (11%) according to the German guidelines. The utilization of both tubes did not reveal any clinically significant deviations (p < 0.05). The values of both tubes resulted in an initial highest deviation of 5.5%, and 6.4% after 48h (both healthy). Comparable highest deviations for initial values in relation to 48h were obtained for both tubes with 5.4% (healthy) and 6.6% (GDM), respectively. The storage of whole blood specimens for 48h showed no significant deviation (10.5%, healthy).

Conclusion: The new glucose tube is suitable for reliable determination of blood glucose diagnosing GDM. The stability of glucose concentration in whole blood specimens stored up to 48h at room temperature has been shown.

Abstract number 0270

Prevalence, pattern, period of onset and predisposing factors of chronic kidney disease in patients with type 1 diabetes mellitus

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Aim: To determine the prevalence, pattern and risk factors of CKD in type 1 diabetes mellitus patients.

Method: Stages of diabetic nephropathy were defined by eGFR (cystatin C based), albumin/creatinine ratio and plasma kidney injury molecule 1 (KIM 1). Fasting plasma glucose (FPG), glycated haemoglobin (HbA1c), and serum C-peptide, islet cell antibody (ICA), and glutamic acid decarboxylase antibody (anti-GAD) were measured. Resistivity index (RI) was by renal Doppler ultrasound.

Results: Ten patients (aged 15-17yr; M: F ratio 3:2; IDDM duration 0.2-9yr) were evaluated. Recent and remote poor glycaemic control were evidenced by FPG (mean 13.55 +/- 5.88mmol/l), and HbA1c (11.72 +/-1.93%) respectively. C-peptide (0.69 +/-0.09ng/ml) was severely low; ICA (43.10 +/- 1.85ng/ml) and anti-GAD (30 +/- 26.23ng/ml) were markedly elevated. Cystatin C was elevated (1.56 +/- 0.78mg/l); the mean eGFR was 56.30 +/- 26.17/ml/min/1.73m2. Six patients had a GFR <60ml/min/1.73m2. One patient had stage 1; 3 had stage 2; 4 had stage 3; 2 had stage 4 but none had stage 5 CKD. GFR decline occurred as early as 2.5 yrs. Three patients had normoalbuminuric renal insufficiency; ACR: 3 microalbuminuric and 3 macroalbuminuric. KIM 1 was elevated (1.8-6.4ng/ml); seven had HDL below desirable levels (0.41-0.78mmol/l); one had increased RI (/>= 0.7).

Conclusion: Moderate CKD is highly prevalent in type 1 DM patients and commences as early as two and a half years of disease. Poor glycaemic control and dyslipidaemia are contributory.

Abstract number 0344

Search for the most promising biomarkers of MODY

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The differential diagnosis of MODY (Maturity Onset Diabetes of the Young) is of great clinical importance. Thus, the challenge is to define cost-effective biomarkers, which could be used to prioritize patients for molecular genetic testing.
The aim of our study was to test jointly two already existing biomarkers: 1.5-AG and hsCRP, for the differential diagnosis of GCK-MODY, HNF1A-MODY and T2DM, T1DM.

We analyzed plasma levels of 1.5-AG (Immuniq kit), and hs-CRP (ErbaMannheim kit) in 111 GCK-MODY patients, 94 HNF1A-MODY patients and 109 T1DM, 102 T2DM subjects. Both biomarkers were evaluated separately using ROC analysis. The analysis were performed using R ver.3.3.

Mean(SD) 1.5-AG levels were: GCK-MODY 11.69 µg/ml (6.49), HNF1A-MODY 5.51 µg/ml (3.16), T1DM 4.4 µg/ml (2.76) and T2DM 7.89 µg/ml (5.23). Levels in GCK-MODY were higher than in other groups in post hoc analysis (p < 0.001 vs. each group). Mean (SD) hsCRP levels were: GCK-MODY 1.64 mg/l (2.8), HNF1A-MODY 0.79 mg/l (1.03), T1DM 1.69 mg/l (3.42) and T2DM 2.79 mg/l (4.26). Levels in HNF1A-MODY were lower than in other groups (p < 0.001 vs. each group).

We calculated threshold values of 1.5-AG designed to reflect maximum sensitivity and specificity; for GCK-MODY versus T2DM, a 1,5AG > 9.2 g/ml gave a 60.9% sensitivity and 73.5% specificity of 58% for identifying the GCK-MODY cases, while 73% of the T2DM cases fall below this cut off. For GCK-MODY versus HNF1A-MODY, 1,5AG > 6 g/ml gave sensitivity of 82.7% and specificity of 64.9% for identifying GCK-MODY, while 65% of HNF1AMODY diabetic subjects had 1.5-AG levels below this cutoff.

By evaluating jointly the two most promising biomarkers of MODY: 1.5-AG and hsCRP we did not show that their combination increases discriminative performance.

4th Joint EFLM-UEMS Congress
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Abstracts selected for presentation during poster walks
Therapeutic drug monitoring and pharmacogenetics of immunosuppressants
Abstract number 0085
Behavior of CD26 and CD28 expression on T-cell populations during the first 6 months after kidney transplantation

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Immune monitoring is a promising new area of research in transplantation medicine aiming at identifying new biomarkers that can foster personalized immunosuppressive therapy. CD26 and CD28 are two surface molecules involved in co-stimulation of T-cells and associated with the risk of rejection early after transplantation. Whereas CD26 is up-regulated in response to T-cell activation, CD28 is constitutively expressed on activated T-cells. We report first results from a clinical study set-up to investigate whether combining these two biomarkers can improve their diagnostic performance. The course of CD26- and CD28-expression on effector (CD4+) and suppressor (CD8+) T-lymphocytes (CD3+) over 6 months post-transplantation was evaluated in 28 kidney transplant recipients (KTR) by FACS analysis. A group of pre-transplant patients (n=49) served as control. Specific expression on memory-T-cells (CD3+CD4+CD65RO+CD26+; CD3+CD4+CD65RO+CD28+; CD3+CD8+CD65RO+CD26+; CD3+CD8+CD65RO+CD28+) was followed too. Friedman-, Mann-Whitney-, and Spearman’s rank correlation tests were used for statistics. All CD26+ cells were also CD28+ but not vice versa. The significant correlation between CD26- and CD28-expression observed was more pronounced in memory-cells. CD26- but not CD28-expression on CD4+ cells was significantly lower post-transplantation compared to pre-transplantation. Both CD26- and CD28-expression on CD4+ cells were stronger suppressed in high-risk KTR (n=8, rigorous immunosuppressive therapy) compared to non-high-risk KTR. CD8+ cells were not similarly affected. The percent CD3+CD8+CD45RO+cells expressing either CD26 or CD28 increased over time post-transplantation but the trend reached significance only in the CD3+CD8+CD45RO+CD28+ population. Both CD26- and CD28-expression reflect changes in the level of immunosuppression/immune activation post-transplantation but differently. This makes the idea to combine them for diagnostic purposes promising.

Abstract number 0191
Rivaroxaban: evaluation of analytical performance

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Background: Despite the fact that coagulation monitoring for direct oral anticoagulants is not routinely recommended, several clinical settings exist when measurement of the anticoagulant activity can be helpful.

The aim of this study was to analyze the analytical performance of chromogenic anti-Xa assay using Rivaroxaban-calibrator and Rivaroxaban-control.

Methods: The chromogenic anti-Xa/RVXN test configuration was implemented on fully automated STA-R Evolution analyzer, using anti-Xa assay (Liquid anti-Xa, Stago, France). The calibration curve of rivaroxaban is of the Log (OD/min)-Lin (concentration) type with working range 25-500 ng/mL. The methods’ intra- and inter-assay precision was evaluated using 2 levels of commercial control (Rivaroxaban Control-1 and Control-2, Stago, France): 5 times on each of 5 days. A coefficient of variation less than 10% was required for imprecision to be acceptable. The external quality assurance sample for Rivaroxaban test was issued by the ECAT (External quality Control of diagnostic Assays and Tests with a focus on Thrombosis and Haemostasis).

Results: Results are expressed as ng/mL of Rivaroxaban from the calibration curve. Intra-assay precision was 3.8% for Control-1 and 2.0% for Control-2 samples. Inter-assay precision was 3.8% for Control-1 and 3.4% for Control-2 samples, representing acceptable imprecision. As an individual performance indicator the Z-score was given in the ECAT survey reports and was acceptable.

Conclusion: The analytical performance of anti-Xa chromogenic assay calibrated for Rivaroxaban is acceptable. This assay can be used in routine practice to determine plasma concentration of Rivaroxaban using drug-specific calibrator and control.

Abstract number 0319

Everolimus steady-state concentration may be related to ABCB1 genotype: possible impact on therapeutic drug monitoring

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The aim of this study was to assess the influence of P-glycoprotein (ABCB1) polymorphism on immunosuppressive drug everolimus (EVE) steady-state trough concentration in heart transplant patients.

Genotypic variants of ABCB1 were determined by PCR/RFLP (3435C→T SNP was analyzed) in a group of 31 patients (26M, 5F; mean age 55 y. (24-76)) after heart transplantation treated with EVE and cyclosporine (CSA, 19%), tacrolimus (TAC, 25%) or mycophenolate mofetil (MMF, 56%). A number of 310 routine EVE samples were measured using specific LC-MS/MS method; mean number of 10 ± 3.4 samples per patient were collected during observation period lasting 22 ± 3.9 months.

The distribution of ABCB1 genotypes in our patients was as follows: 16% of CC, 58% of CT and 26% of TT genotype. A significant influence of co-administered CSA or TAC on EVE concentration was noted thus only a subgroup of 18 EVE-MMF patients (16M, 2F; genotypes: 3 CCs, 9 CTs and 6 TTs) carrying 159 samples were taken into further statistical evaluation. Mean EVE trough concentration was 5.00 ± 1.45, 5.43 ± 1.42 and 5.64 ± 1.69 ng/mL for CC, CT and TT genotypes, respectively (p=0.1231, p=0.0499, p=0.3722 for CC/CT, CC/TT and CT/TT, respectively) however, mean dose-normalized EVE trough concentration was: 2.26 ± 0.79, 3.39 ± 2.00 and 3.07 ± 1.06 L-1x10-3 for CC, CT and TT genotypes, respectively (p=0.0003, p=0.0012, p=0.9903 for CC/CT, CC/TT and CT/TT, respectively).

Significant differences in dose-corrected EVE concentration between CC and the other two genotypes suggested possible impact of ABCB1. The results should be confirmed on larger scale studies involving pharmacokinetic evaluation.

Abstract number 0360

Pharmacodynamic therapeutic drug monitoring of belatacept


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Belatacept blocks CD28-mediated T-cell activation by binding CD86 on antigen presenting cells. No therapeutic drug monitoring of serum levels is recommended for belatacept, because of low pharmacokinetic inter-patient variability. We questioned whether the CD86 competition flow cytometry assay on monocytes could be useful as a tool for pharmacodynamic monitoring of belatacept. CD86-expression was assessed on monocytes of patients treated with tacrolimus or the Less-Intensive regimen of belatacept during a stable or rejection period. One rejected renal graft was stained for CD86.
Before transplantation, flow cytometric analysis of whole blood samples showed that CD86 was expressed on monocytes: median 2029 molecules/cell [1179-4102]. After one dose of belatacept the numbers of free CD86-molecules per monocyte dropped by >85% in all patients (n=20), p=0.0003. In tacrolimus-treated patients (n=20) the expression levels of this co-stimulatory molecule decreased by 33% (p=0.003), less than in belatacept treated patients (p<0.0001). For the entire one year study period the numbers of free CD86-molecules per monocyte remained stable in patients with either belatacept or tacrolimus, irrespective of rejection. In the rejected renal graft, 15-20% of the mononuclear cells still expressed CD86.

The blockade of CD86 on blood monocytes did not differ during rejection, which makes this assay less suitable for TDM to prevent rejection. Even though part of CD86 on blood monocytes and tissue mononuclear cells was not blocked by belatacept, the measurement of free CD86 on blood monocytes will not be instrumental in improving efficacy of belatacept treatment.

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Abstracts selected for presentation during poster walks
Laboratory assessment of kidney function
Abstract number 0099

Evaluation of a new automated microscopy urine sediment analyser - sediMAX conTRUST®

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Background: Urine analysis remains important in the diagnosis and monitoring of urinary tract infections and renal diseases. Manual microscopy is the golden standard, despite poor precision and wide inter-observer variability. This study evaluated the performance of sediMAX conTRUST® (77Elektronika, Budapest, Hungary) as an alternative to microscopic analysis of urine.

Methods: The validation included a precision, carry-over, categorical correlation and diagnostic performance study with manual phase-contrast microscopy as reference method. A total of 260 routine urine samples were assessed.

Results: Within-run standard deviation (SD) of sediMAX conTRUST® was 0.53 RBC/μL at 0.78 RBC/μL with a coefficient of variation (CV) of 6% at 661 RBC/μL. SD was 0.76 WBC/μL at 2.49 WBC/μL and CV was 3% at 411WBC/μL. Between-run CV was 7% for 363 RBC/μL and 12% for 218 WBC/μL. There was no sample carry-over. The analyzer showed good categorical agreement (K ≥ 0.61) with manual microscopy for RBC and WBC counts, moderate (K = 0.41-0.60) agreement for yeast cells, crystals and squamous epithelial cells and bad (K ≤ 0.40) agreement for non-squamous epithelial cells, bacteria and casts. Diagnostic performance was acceptable only for RBC (sensitivity/specificity = 88%/99%), WBC (sensitivity/specificity = 86%/95%) and yeast cells (sensitivity/specificity = 100%/98%). The number of false negative results was acceptable (< 4%) for all elements after the implementation of review rules.

Conclusions: The sediMAX conTRUST® should be used as a screening tool, identifying normal samples. Therefore, adequate review rules should be defined. Manual microscopy is still required in ‘flagged’ pathological samples.

Abstract number 0223

Glomerular filtration rate estimation using β-trace protein: external validation of three equations

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Introduction: Beta-trace protein (BTP) is a low-molecular-weight protein emerging as a novel endogenous glomerular filtration rate (GFR) marker. Different BTP-based equations were proposed to translate plasma BTP levels into estimated GFR (eGFR) in populations with mean measured GFR (mGFR) >40 ml/min/1.73 m2. Here we evaluated three BTP-based equations in a population merely constituting of CKD stage 5 patients (pts).
Methods: Plasma BTP was measured in 665 pts with CKD [CKD stage 3-4 (n=86), CKD stage 5 on HD (n=217), PD (n=83) or without RRT (n=279)] and 45 control subjects (HS) using a nephelometric test (N Latex BTP, Siemens). Three BTP-based eGFR were calculated based on the following formulas: Poge et al GFR = 974.31 x BTP-0.2594 x creatinine-0.647; White et al GFR = 167.8 x BTP-0.758 x creatinine-0.204 (x 0.871 if female); Inker et al GFR = 55 x BTP-0.695 x 0.998age (x 0.899 if female). Using Bland Altman analysis, level of agreement with eGFR MDRD was assessed in the entire cohort. Additionally, agreement with mGFR was studied in CKD stage 5 (average urinary clearance of creatinine and urea, n=298) and CKD stage 3-4/HS (iohexol clearance, n=123).

Results: Among 665 pts (age 59 ± 14 yrs; 63% male, 1% African), mean eGFR MDRD was 13.4 ± 19.5 ml/min/1.73 m2. BTP levels (mg/l) significantly differed between groups (HS 0.33 ± 0.12; CKD 3-4 2.00 ± 0.75; ESRD 4.95 ± 1.89; PD 7.07 ± 2.44; HD 9.40 ± 3.03; p < 0.001) and appeared to be independent of age and gender. Overall, BTP-based equations tended to overestimate eGFR in comparison with MDRD (Table 1 and Figure 1) with bias increasing with higher mean eGFR. In CKD stage 3-4, best agreement with both eGFR and mGFR was found with the Poge's equation. Fair agreement was found between mGFR and the Poge equation in CKD stage 5, but with more overestimation than the MDRD formula.

Conclusion: In the studied population, only the Poge formula, using combination of both BTP and creatinine levels, is accurate enough to be an equivalent of the MDRD formula over the whole range of GFR. BTP-based equations could be valuable in CKD stage 5, but further validation against the gold standard is warranted.

Abstract number 0231

Plasma beta-trace protein predicts mortality in CKD: relation with endothelial dysfunction

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Introduction: BTP, or lipocalin-type prostaglandin D synthase, is a low-molecular weight protein emerging as a novel glomerular filtration rate (GFR) marker. BTP has also been shown to have prognostic value regarding all-cause and cardiovascular mortality in CKD patients. Methods: Baseline plasma levels of BTP in 663 patients with CKD [CKD stage 3-4 (n=86), and CKD stage 5 on HD (n=215), PD (n=83) or without RRT (n=279)] were determined using a nephelometric test (N Latex BTP, Siemens). The cohort was divided in BTP tertiles. Soluble vascular cell adhesion molecule-1 (sVCAM-1) levels were measured in a subgroup of patients (n=355) as a surrogate marker of endothelial dysfunction.

Results: Of the 663 patients studied (age 59 ± 14 yrs; 62% male; eGFR MDRD 8.8 ± 7.5 ml/min/1.73m2), 206 patients (31%) died during a median follow-up of 31 months. Kaplan-Meier survival analysis shows a higher survival probability of the patients with lowest BTP. In multivariate Cox proportional hazard analysis, adjusting for age, sex, presence of cardiovascular disease and diabetes as well as for creatinine, BTP appeared to be strongly and significantly associated with mortality (HR for middle + high vs low BTP 1.92; 95% CI 1.22-2.92). There was a positive correlation between sVCAM-1 and BTP levels (r=0.435, p < 0.001), independent of creatinine levels in multivariate regression analysis (β 0.514).

Conclusions: Plasma BTP levels are associated with mortality in patients with CKD stages. The strong correlation with sVCAM-1, independent of creatinine, might point to endothelial dysfunction as a non-GFR determinant of BTP levels.
Aim: We aimed to compare two automated 25(OH)D assays: enzymatic (Pentra 400, Horiba ABX) and immunochemiluminescence (IDS-iSYS Immunodiagnosticsystems) using pediatric blood samples.

Material and methods: 25(OH)D total was measured in the serum of 100 schoolchildren aged 9 - 11 yrs (45 boys and 55 girls) on Pentra 400 and IDS-iSYS platforms. The anthropometric measurements were conducted and body mass index percentiles were calculated with an online BMI calculator (based on the “OLAF” project).

Results: Both methods demonstrated “poor correlation” (pc = 0.26). The Spearman’s rank correlation has shown a positive “moderate correlation” (rho = 0.47). Mean biases differed when comparing two subgroups of children. The one with the optimal body mass achieved the mean bias 35.3% (LoA: 109.8% to -39.1%), whereas in children categorized as overweight/obese the mean bias was only 2.0% (LoA: 80% to -75.9%).

Conclusions: Assays for 25(OH)D evaluation performed on Pentra 400 and IDS-iSYS showed uneven scores. Both analyzed method are not comparable, partly due to high cross-reactivity of the antibodies used in enzymatic method with C3-epimers of 25(OH)D, and cannot be used interchangeably, especially for evaluation of children vitamin D status. Further comparison study with “gold standard” methods and more age-diverse group is recommended.

Abstract number 0134

Proteomic analysis of the composition of meconium proteins and their classification

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Background: The intrauterine environment, shared genes and postnatal environment can be associated with perinatal complications and long-term health outcomes. Meconium as biological material formed in the fetal intestine exclusively in utero and passed naturally by a neonate may contain proteins which characterise the intrauterine environment. The aim of this study was proteomic analysis of the composition of meconium proteins and their classification by biological function.

Methods: Proteomic techniques combining IEF fractionation and LC-MS/MS analysis were used to study the protein composition in a meconium sample obtained by pooling 50 serial meconium samples from 10 healthy full-term neonates. The proteins were classified by function based on a literature search for each protein in the PubMed database.

Results: A total of 946 proteins were identified in the meconium, including 430 proteins represented by two or more peptides. When the proteins were classified by their biological function the following were identified: enzymatic proteins, immunoglobulin fragments, neutrophil-derived proteins, structural proteins and fetal intestine-specific proteins.

Conclusions: Proteomic analysis of the composition of meconium proteins and their classification can provide information on the physiological and pathological processes during gestation. Better understanding of different biological functions of meconium proteins in the intrauterine environment may help to identify these proteins as biomarkers with impact on the fetal development and further deleterious consequences in older infants and children as well as in adult life.

Abstract number 0213

Presepsin (soluble CD14 – ST) as a new marker for the diagnosis of sepsis in newborns

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Background: CD14 is a glycoprotein expressed on the surface membranes of granulocytes, monocytes and macrophages and serves as a high-affinity receptor for complexes of lipopolysaccharides (LPS) and LPS binding protein. The complex of LPS-LPBP-CD14 is released into circulation by shedding of CD14 from the cell membrane yielding soluble CD14. Plasma protease activity generates also another sCD14 molecule called sCD14 subtype or presepsin, whose increase in the blood of septic patients, is more sensitive marker for the sepsis than procalcitonin, interleukin-6 and increased faster than C-reactive protein, procalcitonin and D-dimers.

Methods: The study comprised 132 newborns, among them 44 (21 with early-onset, 23 with late-onset; 20 preterm, 24 full-term; 25 born by Cesarean section, 19 born vaginally) septic newborns, 38 with early-onset local infections. 20 newborns without infections and 30 eutrophic, healthy, full-term, breast-fed, born vaginally. The presepsin concentration was measured in whole blood samples using the PATHFAST TM
analyzer (Mitsubishi Kagaku Iatron Inc), based on chemiluminescent enzyme immunoassay (CLEIA). The results were obtained within 17 minutes. The serum CRP and PCT concentration were measured with Cobas 6000 analyzer (Roche, Germany). Normality of the data was tested using the D’Agostino-Pearson test. The Mann-Whitney U test, Kruskal-Wallis test and Spearman rank correlation were used. ROC analysis was used to examine the capability of presepsin to sepsis diagnosis.

Results: In septic neonates the mean presepsin concentration (1298.3 ± 541.9 pg/ml) was significantly (p < 0.001) higher than that in control group (391.3 ± 83.6 pg/ml), in newborns with local infections (727 ± 376.2 pg/ml) and in newborns without infections (528.4 ± 178.3 pg/ml). A significant positive correlation (p = 0.0004, r = 0.531) between presepsin and CRP concentrations was observed in septic newborns.

Conclusions: Measurement of blood presepsin concentration is a highly specific and sensitive biomarker for the early diagnosis of neonatal sepsis.

Abstract number 0268

Multiparameter assessment of immunophenotype of leukemic blasts in B-cell precursor acute lymphoblastic leukemia

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Introduction: Determination of immunophenotype of leukemic blasts in B-cell precursor acute lymphoblastic leukemia (BCP-ALL) at diagnosis is crucial for minimal residual disease detection by multicolor flow cytometry. The purpose of the study was the evaluation of expression levels of multiple antigens on blasts in BCP-ALL.

Patients and Methods: The study included 420 pediatric patients treated at the centers of the PPLSG. Bone marrow samples were stained at initial diagnosis using 8-color monoclonal antibody panels. The expression of cIgM, TdT, CD22, CD27, CD19, CD34, CD38, CD10, CD20 and CD45 on blast cells was determined using multicolor flow cytometry technique. The expression levels of the above-listed markers were evaluated with Infinicyt™ software (Cytognos, Spain) as a percentage of positive population with reference to negative population (T-cells, erythroblasts) and normal B-cell precursors.

Results: In BCP-ALL, the homogenous positive (>80% blasts) expression of CD19 (90% cases), CD22 (91% cases), TdT (72% cases) and CD10 (93% cases) on leukemic blasts were most frequently observed. Secondly, the positive homogenous expression of CD34, CD45, CD38 and cIgM was found in 43%, 28%, 56% and 15% of all BCP-ALL cases, respectively. Finally, the homogenous expression of CD27 and CD20 was relatively rare (5% and 3% cases, respectively) among BCP-ALL cases. The differences of expression levels of antigens analyzed in normal and malignant BCP were sufficiently high for patient-specific minimal residual disease monitoring.

Conclusion: Multiparameter flow cytometry is an important tool for characterization of blast heterogeneity at diagnosis in BCP-ALL and for subsequent minimal residual disease monitoring.
4th Joint EFLM-UEMS Congress

Thursday September 22nd

Abstracts selected for presentation during poster walks

Molecular diagnostics in cancer management

Abstract number 0011

Assessment of the performance of specific prostate diagnostic tools in the detection of prostate cancer among Ghanaian men


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This cross-sectional prospective study evaluated the individual and combined performances of specific prostate diagnostic tools in the detection of prostate cancer among 128 Ghanaian men suspected of having prostate cancer (PCa) after having undergone trans rectal ultrasonography guided biopsy of the prostate. The diagnostic tools assessed included; DRE, PSA, and prostate specific antigen density (PSAD). PCa was diagnosed in 21.09% of the patients, of which, 96.3% had abnormal DRE and 37% had abnormal DRE and PSA levels <4.0ng/ml. 40.74% of the patients had their PSA in the range of 20ng/ml-50ng/ml. 77.0% had PSAD higher than 0.15ng/ml/ml. PSA and PSAD were significantly higher (p<0.001) among patients with PCa. PSAD showed a better accuracy (AUC=0.821) followed by a combination of PSAD/DRE/PSA (AUC=0.784) and DRE (AUC=0.780). PSAD had sensitivity and specificity of 77.8% and 72.0% respectively. PSA had a sensitivity of 96.0% and specificity of 30%. DRE had a specificity of 96.0% and a low sensitivity of 48.5%. Increased PSA (OR=11.4), PSAD (OR=9.0) and abnormal DRE (OR=20.0) were significantly (p<0.001) associated with increased risk of PCa. Although, serum PSA has a good specificity as a biomarker, it lacks the clinical specificity for a definitive diagnosis of PCa. On the other hand, DRE had adequate specificity but lacks good sensitivity in reaching a clinical decision of suspected case of PCa. This study has clearly shown that, PSAD gives a better diagnostic accuracy followed by combinations of PSAD/PSA and their DRE as diagnostic markers in the detection of PCa among Ghanaian men.

Abstract number 0296

Detection of EGFR mutations in cell-free DNA from patients affected by lung cancer: preanalytical and analytical aspects

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Lung cancer is the leading cause of cancer death worldwide. EGFR mutations are driver oncogenic mutations against which EGFR tyrosine kinase inhibitors (TKIs) represent an effective treatment. Recently AIOM guidelines indicated cell-free DNA (cfDNA) as a surrogate for the determination of EGFR status in patients without available tumor sample.

Our aim is to evaluate different approaches for the identification of EGFR mutations in cfDNA from lung cancer patients in view of a clinical application. In particular we focused on mutations associated with sensitivity to EGFR TKIs, such as deletions in exon 19 and the mutation p.L858R in exon 21, and on the mutation p.T790M in exon 20, responsible for resistance to treatment.

We considered the preanalytical phase by comparing two methods, manual and automated, for cfDNA extraction: the automatic procedure showed a better yield than the manual.

We compared the analytical performances of two methods based on real-time quantitative PCR (qPCR) with those of a digital PCR (dPCR) approach. From the results obtained on reference standard samples at known percentages of mutated alleles dPCR appeared as the most sensitive method, being capable of detecting L858R mutation and a deletion in exon 19 in a sample containing 0.1% mutated DNA.

Preliminary data on patients affected by lung cancer showed concordant results between the two qPCR methods. Some discrepancies in the mutational status of EGFR were found between tissue and cfDNA.

Upon optimization and standardization of methods, non-invasive analysis of cfDNA might replace biopsy for a precision medicine approach in lung cancer.
Abstract number 0345

Digital PCR as a potential reference method for molecular diagnostics from liquid biopsies

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Advanced, high-accuracy diagnostics measuring key genetic markers are required for the predicted impact of precision/stratified medicine to be realised. However, mechanisms to facilitate metrologically traceable quantification are needed to enable compliance with Clinical Standards (eg ISO 17511 and 15189) and to maximise clinical measurement quality and comparability. The aim of this study was to develop candidate reference methods and materials to determine whether metrological traceability to the SI based on enumeration methods could support genetic detection of cancer mutations. Genetic analysis of cell free nucleic acid tumour markers in blood (liquid biopsies) was the chosen model for this study. Reference panels of materials were prepared containing different quantities of cancer biomarkers (KRAS) in a wild-type background. A protocol was also developed to prepare <200 bp fragments to mimic circulating cell free DNA. Materials were characterised to determine sources of impurities for quantitative methods. Digital PCR (dPCR) was investigated as a candidate reference method for assignment of absolute values to the materials through assessments using different assays, instruments and laboratories. Using the KRAS reference panels' dPCR was demonstrated to be highly reproducible when comparing six instruments and four different assay chemistries. Inter laboratory reproducibility was also high without the use of a calibrator. The use of dPCR for the absolute value assignment of nucleic acid based reference materials will help improve traceability and ensure comparability of molecular measurements. This could support the translation of high accuracy methods in to the clinic and application of precision/stratified medicine to patient care.

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Abstracts selected for presentation during poster walks

Other topics

Abstract number 0016

Development of a structured database system describing clinically relevant drug-laboratory test interactions

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Of all hospital admissions, 5-10% is caused by drug-related adverse effects. In clinical practice, unexplained abnormal laboratory test results are observed regularly. Interactions with medication may explain some of these results, but are currently not easily identified in daily routine. The aim of the Working Group on Drug-Laboratory test Interactions of the Dutch Society for Clinical Chemistry and Laboratory Medicine is to integrate the available information on clinically relevant interactions into a database that facilitates integration of laboratory results and pharmacy records by decision support systems. Candidate interactions between laboratory test parameters and medication were selected from the national database on side effects (Lareb), most prescribed medication in hospitals, most requested laboratory tests, and expert opinion. The interactions were evaluated independently by at least two working group members by systematic literature search. Results were described in a database - including information on effect (nature, extent, incidence), level of evidence, time frame (start, duration), and groups at risk - and summarised in a validation report. An interpretative comment was added to each interaction to inform laboratory specialists or physicians about the possible impact of the interaction. All reports were reviewed, adjusted when needed and finally approved by all working group members. The database and validation reports serve as a national guidebook to enhance the consulting role of the laboratory specialist in advising physicians. Furthermore, the database will be validated in clinical practice to evaluate its use in decision support systems after electronic linkage of the laboratory test results and pharmacy records.
Abstract number 0069

Within-day biological variation and hour-to-hour reference change values for hematological parameters

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Background: Middle and long-term biological variation data for hematological parameters have been reported in the literature. Within day 24-hour variability profiles for hematological parameters are currently lacking. Comprehensive hour-to-hour variability data is however critical to detect diurnal cyclical rhythms, and takes into account the ‘time of sample-collection’ as a possible determinant of natural fluctuation. In this study, we assessed 24-hour variation profiles for 21 hematological parameters.

Methods: Blood samples were collected under standardized conditions from 24 subjects every hour for 24 hours. At each measurement, 21 hematological parameters were determined in duplicate. Analytical variation (CVA), within-person biological variation (CVI), between-person variation (CVG), index of individuality (II) and reference change values (RCV) were calculated. For the parameters with a diurnal rhythm, hour-to-hour RCVs were determined.

Results: All parameters showed higher between-person biological variation (CVG) than within-person biological variation (CVI). Highest between person variation was found for immature platelet fraction (50.8%; 95% CI 38.1-76.2%) and the lowest value was MCHC (3.1 mmol/L; 95% CI 2.4-4.6 mmol/L). Within-subject biological variation varied from 0.4% (0.33-0.42%) to 13.2% (12.1-14.4%) for red cell distribution width (RDW) and immature platelet fraction (IPF), respectively. Six hematological parameters showed a diurnal rhythm.

Conclusions: We present complete 24 hour variability profiles for 21 hematological parameters. Hour-to-hour reference change values may help to better discriminate between random fluctuations and true changes in parameters with rhythmic diurnal oscillations.

Abstract number 0092

Effects of FXa inhibitor apixaban on routine and specific coagulation assays: a European survey

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Aim: FXa inhibitor apixaban is currently indicated for thrombosis prevention in atrial fibrillation and orthopedic surgery and deep venous thrombosis treatment. We aimed to assess apixaban effects on routine and specific coagulation assays with multiple reagents.

Methods: Lyophilized apixaban samples in four concentrations 75, 120, 270 and 300 ng/mL (Diagnostica Stago and Technoclone) were sent to 28 European laboratories with additional five-point calibrators (Technoclone) to 20 laboratories. Laboratories were asked to report the INR, APTT and anti-Xa (with both local heparin and apixaban calibration).

Results: Twenty-one laboratories reported results. Increasing apixaban concentration prolonged APTT and INR only modestly, mostly within the reference interval. Different thromboplastin reagents were similarly insensitive to apixaban, INR values with HemosIL reagent were on average higher than with other reagents (p<0.001). APTT more exceeded the reference range when using SynthasIL (Instrumentation Laboratory) and STA-PTT-A (Diagnostica Stago) reagent than with other reagents (p<0.001). Only 7 (33%) laboratories reported anti-Xa results with apixaban calibration. They were able to measure concentrations accurately: on average 78, 120, 294 and 320 ng/mL, (CV 10.8-16.1%). Heparin-calibrated anti-Xa activity inclined with increasing concentrations, however, it showed large variation (CV 21.1-48.8%).

Conclusions: INR and APTT do not suit to apixaban capture, though laboratories should know the drug effects on their reagents. Anti-Xa calibrated with apixaban quantifies the drug concentration in plasma accurately.

Abstract number 0115

Big differences in primary care request of laboratory tests for prostate cancer in Spain

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To study laboratory tests demand for prostate cancer at a National big scale in Spain and the differences between autonomous communities (CCAA).

Spain is divided in 17 CCAA also divided in Health Departments that cover a geographic area and its population, with a laboratory that attends the needs of every inhabitant. Each participating laboratory was required to obtain patient data from local Laboratory Information Systems, number of prostate specific antigen (PSA) and free PSA (fPSA) tests in year 2014, and to provide organizational data. The request of every test per 1000 inhabitants and ratio of test ratios (fPSA/PSA) were calculated and compared in the different CCAA, with more than 4 participants.

110 laboratories, participated (27798262 inhabitants). 1431678 and 220212 PSA and FPSA were requested, corresponding to 5955780.5€ and 572551.2€. A median of 57.70 and 6.20 requests per 1000 inhabitants were obtained for PSA and fPSA respectively. 10 CCAA had more than four participants. PSA test demand doubled from the least to the most demander CCAA, the same with fPSA request and consequently with the expenses in PSA and fPSA testing per capita.

Primary care requesting of laboratory tests for prostate cancer in Spain continues to increase. The differences in PSA and fPSA testing between CCAA, and the expenses per inhabitant, reflect we are facing a problem of inequity, and inappropriateness. National and regional policies are necessary through inter-departmental and inter-regional communication and cooperation in order to obtain and appropriate request in the use of laboratory tests for prostate cancer.

Abstract number 0121

Within-subject biological variation data obtained from 91 healthy subjects for nine serum enzymes. Project of EFLM working-group on biological variation

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Aim: An EFLM project was established to deliver new biological variation (BV) data, with confidence limits, for nine enzymes.

Method: A cohort of 91 healthy subjects (38 male and 53 female, 21-69 years old ) were bled for 10 consecutive weeks at one of six European laboratories. An equivalent and stringent pre-analytical protocol was followed at each center to deliver the blood samples. Separated serum were stored at -80°C prior to analysis in duplicate within a single run on ADVIA 2400 Clinical Chemistry System (Siemens Healthcare) in San Raffaele Hospital, Milan. Reference materials (frozen sera with target value assigned by a JCTLM laboratory) were analyzed to obtain traceability. The data were subject to outlier analysis prior to CV-ANOVA, to determine the BV estimates with confidence limits. Data elaboration was performed using Excel 2010.

Results: With the exception of lipase: CVI Male = 6.6% (5.8–7.4); CVI Female = 9.6% (9.0–10.5) there were no statistical differences observed between genders in within-subject BV estimates (CVI(95%CI)). Estimates of the CVI for the other eight enzymes were: ALT: 9.7% (9.1-10.4); AST:
Conclusion: The new estimates of CVI presented were delivered using a stringent protocol and modern analytical methods. They are statistically significantly lower than existing published data derived using older analytical methods now considered obsolete. The new BV data deliver lower, and more appropriate, analytical goals for imprecision for the 9 enzymes studied.

Abstract number 0126

Prognostic value of CEA, IGF-1, IGFBP-3 and IL-6 in colorectal cancer patients

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Aim: The purpose of our study was to evaluate the prognostic value of CEA, IGF-1, IGFBP-3 and IL-6 serum levels in colorectal cancer patients. Material and methods: Serum concentrations of CEA, IGF-1, IGFBP-3 and IL-6 were measured in 234 colorectal cancer patients and in 40 healthy individuals. For each person IGF-1/IGFBP-3 ratio was calculated.

Results: Colorectal cancer patients, in comparison to the reference group had significantly higher concentrations of CEA (p=0.002055) and IL-6 (p=0.0000001). There were no significant differences in IGF-1, IGFBP-3 and IGF-1/IGFBP-3 values. There were significant correlations between the concentrations of: CEA vs IL-6 (r=0.269; p=0.0001), IGF-1 vs IGFBP-3 (r=0.5961; p=0.001) and significant reciprocal correlations between: IL-6 vs IGF-1 (r=-0.1595; p=0.015), IL-6 vs IGFBP-3 (r=-0.1384; p=0.034) and IL-6 vs IGF-1/IGFBP-3 (r=-0.1426; p=0.029). Analysing concentrations of the determined factors in respect of tumour stage (I-II vs. III-IV) significantly higher CEA concentration was found in more advanced group of patients, with lack of significant differences in IGF-1, IGFBP-3, IGF-1/IGFBP-3 and IL-6. Univariate analysis showed significant relationships between overall survival, tumour stage (p=0.0000001), CEA (p=0.0000001), IGF-1 (p=0.001316), IL-6 (p=0.000004) and IGF-1/IGFBP-3 (p=0.001369) value. Multivariate Cox regression analysis showed that serum concentrations of CEA, IL-6 and IGF-1/IGFBP-3 value were apart from tumour stage the most important risk factors for poor survival in the studied group of patients with colorectal cancer.

Conclusion: Apart from stage of disease, the independent prognostic factors in colorectal cancer patients are CEA, IL-6 and IGF-1/IGFBP-3 ratio.

Abstract number 0242

Interference in thyroid function testing determination using PEG methodology

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Background: Thyroid function testing (TFT) nowadays is usually performed by immunoassay method. Interference in TFT can affect the interpretation of the results, which may lead to misdiagnosis, inappropriate investigation and management. Polyethylene glycol precipitation (PEG) method is relatively simple and inexpensive method which can be used to determine the interference in immunoassay. Previous studies used a cut-off of recovery percentage of <40% to indicate the presence of interferences. The aim of the study is to evaluate the recovery percentage of post-PEG precipitation in thyroid function testing in normal subjects.

Methods: Sixty-nine healthy subjects who came for health check-up without previous thyroid diseases were included. Obtained plasma samples were tested for FT3, TT3, FT4, TT4, (all four tests used competitive methods) and TSH (sandwich method) using two immunoassay platforms – the Cobas8000 e602 (Roche) and the Architect i1000SR (Abbott). All plasma samples were tested for anti-TPO using the Architect i1000SR. If all TFT and anti-TPO tests were normal, samples were treated with 25% w/v PEG6000 in water and retested for TFT in both analyzers.

Results: From 69 subjects, 40 (20 Male, 20 Female) had normal results in all TFT and anti-TPO tests. Mean±2SD (%) of recovery values for FT3, TT3, FT4, TT4, TSH were 159.1±13, 184.7±31, 183.8±16, 116.4±10, 52.6±12 in Cobas8000 analyzer, and 181.2±30, 133.1±18, 195.4±13, 132.5±15, 19.1±3.

Conclusions: Tests (FT3, TT3, FT4, TT4) used competitive immunoassay had increased recovery values, while sandwich immunoassay (TSH) had decreased recovery values, and each method of TFT had different recovery values after PEG precipitation.
Abstract number 0261

**Catechol-O-methyltransferase – genetic variants in the Polish population**

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Introduction: Catechol-O-methyltransferase (COMT) is one of the major enzymes involved in the metabolic degradation of catecholamines. COMT genetic variations have been associated with risk of chronic pain development, susceptibility to schizophrenia and anorexia nervosa, as well as response to cannabis use. Our aim was to estimate genotype, haplotype and allele frequencies of the essential loci: rs4818, rs4680, rs6269, rs4633, rs165599 of the COMT gene in the Polish population in the context of cannabinoids pharmacogenetics.

Materials and Method: We analyzed DNA samples from 200 unrelated subjects. The genotypes were determined using pirosequencing and analyzed by Haploview software and Court lab-HW calculator.

Results: All 5 polymorphisms were present in our studies group. The allele G in position rs4818 was identified with frequency of 42.9%, allele G in rs4680 - 48.8%, allele C in rs6269 – 42.4%, allele C in rs4633 – 49.2% and allele C in rs165599 – 30.5%. Finally, all genotype and allele frequencies, excluding rs4633 (p < 0.05), were in Hardy-Weinberg equilibrium. Haplotype analysis confirmed strong linkage disequilibrium (D’=1) between rs4818, rs4680 and rs165599.

Conclusions: We demonstrated that the distribution of the investigated COMT gene variants corresponds to the results from the studies obtained for Caucasians. High polymorphism of these loci suggests its importance in the pharmacogenetic and side effects occurrence after cannabis treatment.

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Abstract number 0324

**Comparability of coagulation assays on two Siemens BCS XP automated coagulation analysers**

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Introduction: The aim of our study was to assess the comparability and estimate bias for some routine coagulation assays on two Siemens BCS XP automated coagulation analysers using patient samples.

Materials and Methods: This comparability study was done in accordance with the CLSI EP09-A3 evaluation protocol, using patient plasma (n=40). We have tested the comparability of PT, APTT, fibrinogen, antithrombin, protein C, D-dimer and TT. Additionally, we have tested precision using two commercial control samples at two concentration levels, in triplicate for 5 consecutive days. Bias was estimated by Bland-Altman analysis and Passing-Bablok regression. Acceptance criteria were based on biological variation.

Results: Precision for all tested assays was within the acceptance limits. Bias was within the acceptance limits for all tested parameters, except for PT (ratio) and PT INR (average bias was 7.76% and 5.72%, respectively).

Conclusion: Most of routine coagulation assays tested in our study (APTT, fibrinogen, antithrombin, protein C, D-dimer and TT) are comparable between two Siemens BCS XP automated coagulation analysers. Due to the presence of bias, coagulation analysers BCS XP should not be used for interchangeable PT assay (ratio and INR). As two identical coagulation analysers may not always necessarily produce identical results, laboratories should verify the comparability for all assays and implement evidence-based protocol for interchangeable routine use.

Abstract number 0328

**Intra and inter internal quality control variability in tumour markers**

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Internal Quality Control (IQC) results from different manufacturers assay systems for a individual tumour markers can vary. The IQC ranges chosen by manufacturers may affect distinguishing between healthy and diseased individuals. The European Group on Tumour markers have identified IQC requirements for tumour markers. These include; demonstrating an intra assay variability of < 5% and inter assay variability of <10%. Most laboratories utilise IQC independent from the assay manufacturer to eliminate bias and to ensure independent assessment.
The aim of our study was to identify and compare IQC ranges and IQC precision within and between analyser systems for different third party control materials available for CEA, C125, CA199, CA153 AFP, and PSA tumour markers.

The results highlight that; CEA and PSA have an elevated % CV in Level 1 third party controls utilising the Siemens Centaur System. Level 3 C125 Thermo-Scientific Omniimmune control exhibited a % CV of >25% on the Roche System compared to a result of 10% utilising Biorad level 3 control.

Siemens, Abbott and Ortho Systems all performed badly on Level 1, 2 and 3 Biorad controls with %CV values at >14-29.6%. This was not observed when compared to Thermo-Scientific controls.

Our study highlights the evidence that there is a high degree of variability between assay systems and selected laboratory IQC which may have a potential effect on the quality and reproducibility of patient results in Irish Hospitals.

Abstract number 0340

Analysis of the crude antigen of hymenolepis nana from mice by SDS-PAGE and the determination of specific antigens in protein structure by Western blotting

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Hymenolepis nana, the dwarf tapeworm, is a common cestode of mice, rats and primates including humans. The purpose of the present work was to determine specific protein bands from the sera of mice naturally infected with H. nana. These research results may provide some basic information required for antigen purification studies. Protein bands of crude antigens of Hymenolepis nana were determined by SDS-PAGE and Western blotting. Thirty Swiss albino mice were allotted into two groups of 15 each as positive (infected with H. nana) and negative (non-infected with H. nana) groups. The natural infections of H. nana and other helminths were determined by centrifugal flotation of faeces. After bleeding, the mice were necropsied and their guts were examined for H. nana and other intestinal helminths. Sera from mice were tested by Western blotting and the bands obtained from positive and negative groups were compared. The specific protein band for H. nana infection was determined to be 24 kDa.

4th Joint EFLM-UEMS Congress

Friday September 23rd

Abstracts selected for presentation during poster walks

Dyslipdemia: New clinical concepts and diagnostic tools

Abstract number 0157

Effect of statins on apolipoprotein E concentration in non-dialysed patients with chronic kidney disease

Agnieszka Ćwiklińska, Monika Cackowska, Ewa Wieczorek, Agnieszka Kuchta, Barbara Kortas-Stempak, Anna Gliwińska, Kamil Dąbkowski, Maciej Jankowski

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HMG-CoA reductase inhibitors (statins) reduce cardiovascular mortality in non-dialysed patients with chronic kidney disease (CKD). This effect is probably not dependent on changes in cholesterol level. Apolipoprotein E (apoE) is generally considered atheroprotective, but there is evidence that increased apoE level correlates with increased cardiovascular disease risk. The aim of this study was to assess the effect of statins on concentration of apoE in serum and its distribution between high density lipoprotein (HDL) and apoB-containing lipoproteins in CKD patients. Adult non-dialysed CKD patients (n=84; age 67 +/- 12; serum creatinine 1.70 +/- 0.56 mg/dl) treated at the Nephrology Clinic of the Medical University of Gdansk were recruited to the study. 47% of patients received statins: 6% - rosvuastatin, 13% - simvastatin, 28% - atorvastatin. Blood was taken after an overnight fast. ApoE was determined in serum (total apoE) and in HDL fraction (HDL-apoE) after precipitation of apoB-containing lipoproteins with heparin and manganese chloride. ApoE in apoB-containing lipoproteins (non-HDL apoE) was calculated from the difference between total apoE and HDL-apoE. Patients receiving statins had lower total apoE and non-HDL apoE concentrations on average by 15% (total apoE median: 3.57 vs 4.17 mg/dl, p=0.022; non-HDL apoE median: 2.25 vs 2.65 mg/dl, p=0.033; patients
receiving and not receiving statins, respectively). HDL-apoE concentration did not differ statistically significantly (medians: 1.20 vs 1.42 mg/dl, \( p = 0.185 \)). Changes in apoE serum level through decreasing apoE in apoB-containing lipoproteins under the influence of statins can contribute to the reduction of cardiovascular risk in CKD non-dialysed patients.

4th Joint EFLM-UEMS Congress

Friday September 23rd

Abstracts selected for presentation during poster walks

New diagnostic tools in infectious diseases

Abstract number 0002

Application of machine learning methods in analysis of MALDI-TOF spectra to generate rapid and accurate strain typing of MRSA

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Specific aim of the study: To provide more rapid and accurate strain typing of MRSA.

Background: Methicillin resistance Staphylococcus aureus (MRSA) is one of the most important nosocomial pathogens. A real time infection control and investigation depends on rapid and accurate strain typing. However, the DNA based typing methods are either time-consuming or cost-ineffective. Matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) is an alternative tool for presumptive report of strain typing.

Methods: MALDI-TOF mass spectra were analyzed for 125 clinically-obtained MRSA isolates. The strain types of the isolates were determined by multilocus sequence typing (MLST). A feature selection process by decision tree (DT) induction algorithm was applied to select robust spectral peaks for designing discrimination models for strain typing. Hierarchical clustering was introduced to analyze intensity of characteristic peaks. Multi-class classification strategy was applied to generate classification models based on various machine learning (ML) methods (decision tree (DT), support vector machine (SVM), and k nearest neighbor (KNN)). The accuracy of the models were compared.

Results: ST 5, ST 45, ST 59, and ST 239 were the major MLST types. To design typing classifiers, nine spectral peaks were selected (m/z value: 1695, 2066, 2451, 2978, 3176, 3891, 4074, 4813, and 6550). For multiclass classification, all the models generated accuracy more than 0.88. No significant difference found between the ML methods.

Conclusion: ML methods could serve as a rapid and accurate tool to provide preliminary strain typing information of major MRSA lineages on the basis of MALDI-TOF spectra.

Abstract number 0087

Therapeutic drug monitoring of meropenem, linezolid and cefepim in severely ill patients on intensive care units (ICU) of the Klinikum Stuttgart

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Altered pharmacokinetics (PKs) in ICU patients with severe infections affect drug exposure which may lead to sub-therapeutic or toxic concentrations of antimicrobials when standard doses regimens are used. In addition, under-exposure bears the risk to yield antimicrobial concentrations inside the so called mutant selection window which is expected to enrich resistant mutant subpopulations. Therapeutic drug monitoring (TDM) of meropenem (MP), cefepim (CP), and linezolid (LZD) by HPLC-UV has been established in the Klinikum Stuttgart for patients with septicemia who were treated with continuous infusion of these antimicrobials. Based on the plasma concentrations doses were adjusted to reach concentrations 4 times (2 times for LZD) above the minimal inhibitory concentration (MIC) either derived from the literature or based on resistance testing of the isolates from patients. TDM was performed in 248 patients (758 blood samples) between March 2014 and 2016. Dose adjustments were required in 37 % with MP, 55 % with CP and 52 % with LZD. Doses were increased in 38 %, 50 %, and 9 % for MP, LZD, and CP respectively. Doses were decreased in 55 % (MP), 50 % (LZD), and 90 % (CP). For MP other interventions such as the adjustment of the infusion rate were performed in 7 %. In conclusion TDM of MP, LZD, and CP revealed the need of dose adjustments in about 40 % of...
Immature platelet fraction (IPF) as a new biomarker of inflammatory changes of different origin?

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IPF - immature platelet fraction is a new haematological parameter representing a fraction of newly formed, immature platelets which reach the final, mature form in circulation within 12 days. Availability of tests for haematological analysers is still very limited, nevertheless some data suggests that it might reflect the inflammatory process in the body. The aim of the study was to evaluate the relationship between IPF and other generally accepted laboratory parameters of inflammation in hospital patients. Material and methods 959 CBC with fluorescence method for platelet count including separate results for IPF(%) were analysed on Sysmex XN-2000, serum CRP was measured by immuno-turbidimetry, PCT by enzymofluorescence. No clinical information was available. Data were divided as follows: 1. All indices within normal range, n = 246 2. All indices moderately elevated, n = 45 3. WBC in 5 ranges from <0.2 to >60 x 10³/µl, n = 959 4. Neut. in 3 ranges from <1.9 to >8.0 x 10³/µl, n = 741 5. CRP in 4 ranges from <50 to >200 mg/l, n = 759 6. PCT in 4 ranges from <0.5 to >200 ng/ml, n = 217 Results and conclusion. A significant difference in IPF was found between group 1 and 2 (5.72 ± 4.25%, versus 8.22 ± 6.36%). IPF also showed a significant increase parallel to WBC in all ranges and was lowest in Neut. <1.9 10³/µl. Otherwise a general tendency to increase IPF% along with other parameters of inflammation was observed. In conclusion IPF% can be considered a promising laboratory indicator of inflammation.

Diagnosis of autoimmune disease

Trends in primary care celiac disease serological markers request in Spain

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Celiac disease (CD) prevalence varies considerably, is increasing and remains undiagnosed. The study compares CD serology markers requested by General Practitioners (GPs) over time and in different Spanish autonomous communities (CCAA).
In 2010 a call for data was posted on the Redconlab website, and via email in the 2012 study. In 2014, the dissemination of the questionnaire was also addressed through a LinkedIn group. Spanish laboratories willing to participate were invited to fill out an enrollment form and submit their results online. Numbers of anti-tissue transglutaminase (anti-tTG) and deaminated gliadine peptide IgA antibodies (anti-DGP), requested by GPs for the year 2010, 2012 and 2014 at different Health Departments (HD) across Spain were reported in the three studies. Each participating laboratory was required to deliver data from local Laboratory Information Systems Patient’s databases and provide organizational data. In the 2014 edition HD were grouped in CCAA when more than 4 participants. Number of anti-tTG and anti-DGP requested per 1000 inhabitants, and ratio of both tests request (anti-DGP /anti-tTG) were calculated. 37, 76 and 110 laboratories, participated consecutively in the three editions. The request of anti-tTG increased along years. Anti-DGP demand was maintained in the first two editions, and decreased in the third. The indicator that relates to both test request (anti-DGP /anti-tTG) diminished, and was lower in 6 CCAA when compared to the others.

There is a need of inter-regional cooperation to develop strategies to optimize the use of CD laboratory tests.

**Abstract number 0316**

Validation of faecal calprotectin determination with the DSX system analyser

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Objective: Calprotectin is a marker for intestinal inflammation that allows for a non-invasive screening for inflammatory bowel disease (Crohn’s disease and ulcerative colitis) that differentiates it from functional disorders.

The measurement procedure is verified by ELISA (Bühlmann fCAL, Palex) with the DSX System analyser (Dynex technologies). This method uses a monoclonal detection antibody and has a greater range of linearity: the upper limit of detection increases to 1800μg/g, compared to 500μg/g using the previous method (ELISA Calprest, Eurospital, Alere).

Methods: The imprecision was studied by checking the results of the controls against the assigned values and that they didn’t exceed quality requirements set by the supplier (CV\% ≤4 for intra-assay precision; CV\% ≤15 for inter-assay precision). The results with the above method were compared via repetitions of 44 samples.

Results: The analysers met the established quality requirements for imprecision in the initial study and during the follow-up period of four months. The new method provides higher values compared to the previous method, mainly for values greater than 200μg/g. One reason was the range of linearity of the old method, in which values between 200 and 500μg/g were located near the saturation zone.

Conclusions: The new method, having a greater range of linearity, allows for better classification of results: a larger number of results are classified in the categories of 50-200μg/g and >200μg/g. In these, additional studies are recommended to confirm an inflammatory bowel disease, thereby decreasing the number of undiagnosed cases, which is advisable for a screening technique.

**4th Joint EFLM-UEMS Congress**

**Friday September 23rd**

**Abstracts selected for presentation during poster walks**

**Detecting age-related changes**

**Abstract number 0061**

Age related vitamin D deficiency and autoimmune thyroid diseases

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Vitamin D deficiency has become a common problem in the general population. Some evidence pointed out that it plays significant role in reducing the incidence of autoimmune diseases, especially autoimmune thyroid diseases (AITD). The aim of our study was to investigate age related relationship between vitamin D deficiency and AITD.
Serum values of total vitamin D and TSH were measured in 506 randomly chosen ambulatory patients not taking vitamin supplements. 342 patients were tested for antithyroid autoantibodies. All tests were performed by electrochemiluminescence immunoassays on e601 Roche analyzer. Patients were divided in two groups based on their age. Vitamins D deficiency was diagnosed at levels lower than 30 nmol/L, hypothyroid were considered patients with TSH levels above 4.5 mIU/L.

Group of patients aged ≥40 years had significantly higher incidence of vitamin D deficiency, hypothyroidism and thyroid autoimmunity compared to <40 years group (p<0.05). In older group patients with positive thyroid antibodies had significantly higher prevalence of vitamin D deficiency compared to antibody negative ones (24.3% vs. 10.6%, p<0.05). There was no such relationship in younger group (18.4% vs. 17.4%). Similarly, there was higher number of patients with hypothyroidism coupled with vitamin D deficiency in ≥40 years group compared to patients younger than forty years (60.6% vs. 35%, p<0.05).

Our results indicate that patients with vitamin D deficiency are more likely to have thyroid function disorders if they are older. Whether supplementation with high doses of this vitamin has preventive or therapeutic effect is subject to work on in further studies.

Abstract number 0301

Geriatric reference values in Belgium based on an indirect approach using patient data

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Reference values for routine clinical chemistry and hematology parameters are typically based on measurements in healthy adults of ≤60 years. However, since the population of senior citizens is continuously growing, there is a clear need for age-adjusted reference values for interpreting lab results. Establishing this by a direct approach is difficult due to the challenge of recruiting a sufficient number of healthy old age volunteers. Therefore, we aimed to establish geriatric reference limits using an indirect method. We collected patient data from 2015 (n = 11432 to 25989) and categorized the data according to gender and age (above 60, using 5 year intervals). Only the first result per patient was included. Samples from the emergency department, intensive care unit, and hemolyzed serum specimens were excluded. Reference limits were estimated with the Batthacharya regression method in each population, using a Gaussian or a Gamma-distribution (depending on skewedness of the dataset). The most prominent changes were an increase in serum creatinine and urea due to declining kidney function. Further, a decrease in albumin was observed. Even though no age-related differences in reference limits for AST and ALT were observed, an increased upper reference limit for CK and LDH was noted between 60 and 79 years. Similar changes were found in studies using direct methods for estimating the reference limits. For hematology parameters, an increased upper reference limit for hemoglobin,blood cell count was observed. The results of this study confirm the need for each laboratory to verify its reference values for geriatric patients.

4th Joint EFLM-UEMS Congress

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Abstracts selected for presentation during poster walks

Clinical applications of genome sequencing

Abstract number 0149

Identification of novel regulators of adipogenesis by RNA-sequencing

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It is generally accepted that the regulation of adipogenesis prevents obesity. However, the mechanisms controlling adipogenesis have not been completely defined. Much of our knowledge of the molecular cascade regulating adipogenesis has come from transcriptome profiling data of adipocyte differentiation generated by DNA Microarray experiments. However, expression microarrays are limited due to a high background signal, the need for known template sequences, variabilities in probe hybridization and a fairly limited dynamic range. Hence, it is a plausible that important genes involved in adipogenesis are expressed below the reliable detection levels of microarrays.

In contrast, deep RNA sequencing (RNA-seq) is a recently developed approach for transcriptome profiling, where the RNA/cDNA species
within a sample are sequenced, that does not suffer from the limitations described for Microarrays. Our goals were to use RNA-seq (i) to identify novel regulators of adipogenesis throughout 3T3-L1 preadipocyte differentiation and (ii) to compare sequencing data to results obtained from Microarrays.

Transcriptome profiling of adipocyte differentiation has improved our understanding of the molecular mechanisms of adipogenesis. Although microarrays have been instrumental in this regard, it is clear that these tools detect an incomplete set of DEG. Therefore the RNA-seq approach can be used to supplement these prior technologies, which could help identify novel target genes involved in adipogenesis in disease.

Abstract number 0173

Genetic screening of medullary thyroid cancer in patients from Algeria

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Introduction: Medullary Thyroid Carcinoma (MTC) can occur in hereditary (25%) or sporadic form (75%). In the hereditary forms, MTC is the major component of the Multiple Endocrine Neoplasia type 2 (MEN 2). MEN 2 is caused by autosomal dominant RET proto-oncogene mutations. Early prophylactic total thyroidectomy before the development of MTC is the only curative treatment.

The aims of this study:
- Determine the frequency and the localization of the detected RET proto-oncogene changes in MTC case index and theirs relatives and to compare them with the data of the literature.
- Present the phenotype–genotype correlation in Algerian MEN2 families.

Patients and methods: DNA was extracted from the peripheral blood lymphocytes of a total of 40 persons, including 25 MTC probands and 15 of their unaffected kindred’s. Exons 8,9,10, 11, 13, 14, 15 and 16 of the RET gene were amplified by PCR and sequenced. Informed consent was obtained from all subjects.

Results and Discussion: The C634Y RET exon11 germline mutation was detected in 8% of our MTC index cases and in 46.66% of their relatives. In relatives G691S and S904S polymorphisms identical to those of MEN2 index cases were found but in the homozygous state, suggesting that this haplotype has a modifying effect on the age of onset of MTC in MEN2A.

In sporadic MTC, the exon 11 G691S SNP, was strongly present. Several studies have shown that this SNP is associated with sporadic MTC predisposition. In our patients, the C634Y mutation was significantly associated with the presence of pheochromocytoma.

4th Joint EFLM-UEMS Congress

Friday September 23rd

Abstracts selected for presentation during poster walks

Point-of-care testing: Methodology and quality

Abstract number 0040

Appropriateness of different analytical performance specifications for glucose meters long term monitoring in a healthcare setting

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Aim: To evaluate the impact of different analytical performance specifications on the degree of accomplishment of the internal quality control long-term monitoring. Material and methods: We daily performed quality control material at low (QC1) and high (QC2) concentration levels (QC1: 45 mg/dL and QC2: 299 mg/dL) on six glucose meters (Accu-check Performa; Roche) managed in a tertiary hospital Blood Drawn Unit.
We calculated monthly imprecision (coefficient of variation) and bias for each device during 15 months, comparing these results with different analytical performance specifications (APS). Different sources for APS were: ISO 15197, ADA, FDA, State-of-the-art: CLIA, Rilibak, SEKK (Czech EQAs), Spanish Minimum Consensus Performance Specifications (SMCS) and Biological Variation (BV). Results: Results are expressed as percentage of results outside the APS / total of results (90 imprecision and bias estimations for each QC level derived from a total of 2700 QC results). Imprecision: ADA and FDA (APS = 5%): QC1 (1.5%), QC2 (1.5%) BV (APS = 2.8%): QC1 (55.4%), QC2 (50.0%) Bias: ISO 15197 (15% if 100 mg/dL), FDA (APS = 15%), CLIA (APS = 10%), Rilibak (APS = 11%), SEKK (APS = 9%) and SMCS (APS = 11%): QC1 and QC2 (None) BV (Desirable bias = 2.3%): QC1 (33.8%), QC2 (72.3%) Conclusions: There are discrepancies when applying different APS, especially when selecting BV. This should be considered before establishing the appropriate APS for QC management according to the intended purpose and the population served. If glucose meters are used for self-monitoring, imprecision should be the main focus in order to assure that the analytical variation is lower than the subject inherent variation.

Abstract number 0066

Verification of the accuracy of three glucose point-of-care testing (POCT) devices for their use in a hospital setting

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Background: Inaccuracy in glucose POCT measurement can lead to inappropriate therapeutic decisions. Here we evaluated the performance of three POCT glucometers by comparing results to those by an automated system traceable to higher-order references.

Methods: 31 heparinized venous blood samples were collected and assayed in duplicate for whole blood glucose concentrations with Roche Accu-Check Compact Plus, Nova Biomedical NovaPro and OKBiotech OKmeterDirect glucometers. All systems were calibrated to report plasma-equivalent results. Samples were then centrifuged and plasma glucose was immediately determined by the hexokinase assay on Abbott Architect c16000 platform. The traceability of Abbott assay was checked by comparison with the hexokinase reference procedure performed on three samples. POCT performance was evaluated according to CLSI POCT12-A3 criteria \( \text{(max 5\% of results } > \pm 12 \text{ mg/dL (for reference results } < 100 \text{ mg/dL) or } > \pm 12.5\% \text{ (for reference results } \geq 100 \text{ mg/dL)})} \) and consensus error grid (CEG) analysis.

Results: The Abbott assay was perfectly standardized (mean bias, 0.13%). Sample glucose concentrations were from 62 to 326 mg/dL, with haematocrit spanning from 0.27 to 0.58 L/L. Average CV on duplicates was 2.8% Roche, 3.3% Nova and 5.9% OKBiotech. All meters gave more than 5% of results (Roche 19.4%, Nova 16.1% and OKBiotech 22.6%) outside the CLSI criteria. However, all results, except two borderline values for OKBiotech, were within the low-risk zone according to CEG.

Conclusions: By using CLSI acceptability criteria, the evaluated glucometers were not accurate enough for clinical use. CEG analysis suggests, however, that this inaccuracy would not have any significant impact on patient outcome.

Abstract number 0298

Assessment of strep a point-of-care testing performance through external quality assurance (EQA) scheme: results of a 6-year study period (2009-2015)

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Group A streptococcus is a leading cause of pharyngitis and several commercial rapid tests are available for POC testing. The use of point-of-care (POC) diagnostic tests is increasing in both developing and developed countries. Rapid antigen-based tests are commonly performed by non-laboratory trained staff in primary and urgent care settings to help diagnosis. External Quality Assurance (EQA) schemes have also become more available to evaluate the POC testing quality. Performance of the POC tests was reviewed and analyzed retrospectively using EQA data from rounds organized during years 2009-2015. Labquality Oy has organized four EQA rounds annually with approximately 500 laboratory participants from several European countries in each round. Excellent specificity was observed for the different commercial POC tests, number of false positive results is less than 1%. Sensitivity on the other hand has been a challenge during the years. Number of false negative results has fluctuated between rounds, but as high as 255/502 (51%) false negative results have been reported from a low positive sample (2x106 bacteria cells/ml). Interestingly, the analysis of the results also indicates the importance of the training and qualification of the test performer. Results show a notable difference in the accuracy of the low positive results based on the professional skills of the person performing the test. Our study results emphasize the importance of the EQA programmes to be used in evaluating point-of-care testing quality.
Dementia genetic and environmental risk factors in a Tunisian population

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Dementia is a multifactorial idiopathic pathology caused by clinical, environmental and genetic factors. Hence its etiology is still unknown. We aimed to evaluate the main risks and protective factors associated with this disease.

We enrolled 122 dementia patients (diagnostic criteria: DSMIV and CIM10) and 190 controls. Data concerning clinical and biological parameters, life style and dietary habits were collected. Genotyping of the 9 studied polymorphisms (PON1-rs662A>T, PON1-rs854560A>G, ACE-I/D-rs1799752, ACE-rs4343G>A, APOE-rs7412C>T, MME-rs701109G>A, IDE-rs1887922C and ECE1-rs213048) were performed by PCR-RFLP. Haplotypes (combination of the respective polymorphisms alleles) were estimated using SNPAnalyzer v2.0 software and biostatistical analysis were conducted on SPSS v20.0.

After binary logistic regression, we noted that factors significantly associated with dementia were hyperhomocysteinemia (OR=5.47; p=0.001), smoking (OR=4.49; p=0.001), hypertension (OR=6.78; p=0.002), Diabetes (OR=4.89; p=0.003), stroke history (OR=5.98; p=0.001), Chronic Kidney Disease (OR=4.69; p=0.001) and high education level (OR=0.08, p=0.002), urban habitat (OR=0.30; p=0.032), currently or formerly active professional life (OR=0.20; p=0.016), consumption of fish (OR=0.21; p=0.012), olive (OR=0.18; p=0.015), curcuma (OR=0.10; p=0.026), coffee (OR=0.20; p=0.021) and black chocolate (OR=0.10; p=0.015).

Except of PON1-rs662 G (OR=1.01; p=0.952), all the studied polymorphisms were significantly associated with dementia, PON1-rs854560 T (OR=1.61, p=0.004), ACE-I/D-rs1799752 D (OR=1.65, p=0.003), ACE-rs4343 A (OR=2.06, p=0.001), APOE-rs429358 C (OR=5.37, p=0.001), MME-rs701109 A (OR=2.21, p=0.001), IDE-rs1887922 C (OR=1.65, p=0.003) and ECE1-rs213048 G (OR=1.62, p=0.003). Four haplotypes appeared to be significantly associated with dementia: ATDGTCGCA (OR=1.65, p=0.001), AADGTCGCA (OR=0.18, p=0.001), APOE-rs7412C (OR=2.06, p=0.003), ACE-rs4343 A (OR=5.37, p=0.001), MME-rs701109A (OR=2.21, p=0.001), IDE-rs1887922 C (OR=1.65, p=0.003) and ECE1-rs213048 G (OR=1.62, p=0.003).

According to our results, a healthy life style and dietary habits mentioned above are recommended to prevent dementia, especially for risk alleles and haplotypes carriers.

Abstract number 0229

Neuron-specific enolase and neurological prognosis in postanoxic coma

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Aim: To study relationship between neuron-specific enolase (NSE) and neurological prognosis of patients in postanoxic coma as a result of a cardiopulmonary resuscitation (CPR), and to estimate values that indicate poor outcome.

Methods: 129 patients that undergone CPR are included. Demographic data were registered, duration of resuscitation, lactate concentration, therapeutic hypothermia (TH) and presence/absence of somatosensory evoked potentials, as well. NSE was measured at 72h post-CPR by immunoassay (Modular E170, Roche Diagnostics). Poor outcome was defined as a Cerebral Performance Category of 3-5. Univariate logistic regression was used for NSE and each control variable, and then a multivariate model was established. ROC was performed, area under the curve (AUC) was calculated and cut-off for NSE allowing classification of patients according to neurological outcome.

Results: The multivariate model, adjusted by lactate concentration, indicated that the association between neurological outcome and NSE remained significant (odds ratios: 1.07 [95% CI: 1.02 to 1.12]; p = 0.002).

In patients undergoing TH, NSE AUC was 0.848 (95% CI: 0.768 to 0.928) and in noTH was 0.956 (95% CI: 0.889 to 1.000). The cutoff values predicting unfavorable outcome, with a specificity of 100%, is 43.4μg/L in TH and 32.4μg/L in noTH, with a sensitivity of 69.2% and 84.6%.
Conclusions: NSE concentrations at 72h have good diagnostic utility in assessing neurological outcome of patients in postanoxic coma. The risk of evolving into a poor prognosis is increased by 7% per 1 ug/L NSE. NSE concentrations higher than 43.4 and 32.4 μg/L in patients undergoing TH and noTH, are indicative of poor neurological outcome.

4th Joint EFLM-UEMS Congress

Thursday September 22nd

Posters

Laboratory biomarkers of cardiovascular disease

Abstract number 0004

Serum asymmetric dimethylarginine levels in patients with masked hypertension

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Aim: There is increasing evidence that elevated levels of the endogenous NO synthase inhibitor, ADMA may contribute to endothelial dysfunction and lead to pathogenesis of several diseases. The aim of this study was to investigate serum ADMA levels in patients with masked hypertension.

Methods: For serum ADMA measurement, serum samples were collected from 40 normotensive, 28 masked hypertensive and 36 hypertensive patients. 100 μL of internal standard (d7-ADMA) in methanol were added to 200 μL of serum and centrifuged at 13,000 rpm for 10 minutes to remove the precipitated proteins. The supernatant was collected and dried under a nitrogen gas flow at 60°C. Derivatisation step was performed dissolving the dried extract in 200 μL of a freshly prepared butanol solution containing 5% (v/v) acetyl chloride and kept at 60°C for 20 minutes. The solvent was removed by evaporation under nitrogen flow at 60°C. The derivatised samples were dissolved in 100 μL of water–methanol (90:10, v/v) containing 0.1% (v/v) formic acid and 40 μL was injected into the UPLC column for chromatography in ABSCIEX API 3200 system.

Results: Serum ADMA levels were significantly higher in hypertensive [0.301 (0.13-0.57)] and masked hypertension group [0.326 (0.13-0.64)] compared to controls [0.215 (0.06-0.29)] µmol/L. (p < 0.001; p = 0.008, respectively). There was no significant difference between hypertension and masked hypertension group for serum ADMA levels (p = 0.383).

Conclusions: Serum ADMA may play a role in both the pathophysiology and screening of hypertension but not useful to identify the transition from masked to hypertension.

Abstract number 0013

Lipoprotein associated phospholipase A2: a surrogate marker of coronary artery disease in diabetic patients

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Background: The aim of this study was to explore if LpPLA2 can be established as a surrogate marker of CAD in diabetic patients and compare it with other established markers like hs-CRP.

Methods: Sixty individuals with angiographically proven CAD and 30 healthy individuals matched for age & sex were studied. CAD patients were divided into two groups based on presence (n=30) [Group I] and absence (n=30) [group II] of type 2 diabetes mellitus (DM). The serum levels of LpPLA2, hs-CRP were measured by ELISA.

Results: Both groups of CAD with and without DM had significantly higher levels of LpPLA2 (Group I 408.48 +/- 38.96 ng/ml, Group II 272.88 +/- 34.21ng/ml respectively) and hs-CRP (Group I 10.61 +/- 1.34 mg/l, Group II 5.75 +/- 2.59 mg/l respectively) when compared with healthy control subjects (LpPLA2 =200.82 +/- 20.97ng/ml & hsCRP=1.89 +/- 1.34mg/l) [p<0.001]. LpPLA2 levels between the two CAD groups were highly significant(p<0.001), levels being maximum for CAD with type 2 diabetes (Group I) which could be due an increase in its substrate
sLDL and oxidised LDL in DM. Angiographic clinical vessel score was done for all patients and was higher in patients of CAD with DM. LpPLA2 levels correlated strongly ($r=0.763, p<0.001$) with the angiographic clinical vessel score in diabetes patients with CAD while hsCRP has moderate correlation with the vessel score ($r=0.475, p<0.01$).

Conclusion: Measurement of LpPLA2 may be considered as a surrogate marker for better prediction of cardiovascular risk in diabetes patients.

Abstract number 0034

Are there an association between higher RDW levels and serious cardiac disease?

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Background: The aim of this study was to determine the relation between red blood cell distribution width (RDW) and N-terminal pro-brain natriuretic peptide (NT-proBNP).

Methods: A descriptive cross-sectional study was carried out. A total of 4338 patients were evaluated and NT-proBNP and RDW were performed. A cutoff of 150 pg/mL was used to establish the difference between cardiac and no cardiac disease and one of 800 pg/mL to evaluate severity. A t-test was used to evaluate the relation between both variables. Statistical analysis was performed using STATA 13.

Results: NT-proBNP mean results were 6107 pg/mL (95% CI: 5596 to 6618 pg/mL) and RDW mean was 14.96 % (95% CI:14.9 to 15.0 %). The mean age of the sample was 81.74 years old. There were 48.40% of women and 51.6% of men.

Patients with cardiac pathology have RDW median 0.81 % higher than non-cardiac origin. (95% CI: 0.598 to 1.026%) with significant differences ($p<0.001$). RDW media was increased in patients with severe disease according to their NT-proBNP levels. Patients with higher RDW values (more than reference intervals) present 1.1 more risk of high NT-proBNP levels, associated with severe disease. (95% CI: 1.06 to 1.13).

Conclusion: There is an association between high NT-proBNP levels and high RDW values. RDW can be useful for determine severity in cardiac disease.

Abstract number 0044

Frequency of the G20210A transition in the prothrombin gene in patients with peripheral artery disease – study in east Algerian population

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Background: Poort and al (1996), Have identified a new abnormality of clotting associated with a thrombotic tendency: G20210A transition in the 3’untranslated region of the prothrombin gene. The role of this mutation in arterial disease is controversial with conflicting results. Available evidence suggests that the G20210A mutation is not a major risk factor for arterial thrombosis. However, it may interact with other environmental and genetic risk factors in promoting arterial thrombosis.

The aim of our study was to estimate the prevalence of this mutation in peripheral artery disease’s patients and to establish a possible association between peripheral artery disease (PAD) and G 20210A prothrombin gene mutation.

Methods: Genomic DNA from 83 cases and 73 healthy controls was isolated from EDTA blood samples using salting out procedure. Presence of prothrombin G20210A mutation was checked by real-time PCR.

All patients and controls gave their informed consent.

Results: The frequency of prothrombin G20210A mutation showed 2.4% in PAD subject, 97.6% were carriers of the GG wild genotype.

In the control group, the frequency of the mutation was found in 1.4% of cases. The other 72 control subjects were carriers of GG genotype with a prevalence of 98.6%.

No homozygous mutated genotype AA was found both in patients and controls.

The frequency of the mutated allele A is only about 1.20% in cases and 0.68% in controls.

Conclusion: In our study, no association between PAD and prothrombin G20210A mutation was detected. Our results agree with some studies but not with others.
Abstract number 0045

Is there a correlation between C677T MTHFR polymorphism and the incidence of peripheral artery disease in east Algerian population?

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Introduction: MTHFR, is a key enzyme in catalyzing 5,10- methylenetetrahydrofolate into 5-methyltetrahydrofolate. A missense mutation of MTHFR that converts alanine to valine (C to T substitution at nucleotide 677) encodes a thermolabile enzyme with lower specific activity. The MTHFR C677T polymorphism as a risk factor in peripheral artery disease (PAD) has been suggested, but direct evidence from genetic association remain inconclusive. The aim of this study is to analyze the prevalence of the MTHFR C677T gene polymorphism and to examine the possible association between PAD and MTHFR gene mutation in PAD patients and to compare them to controls.

Methods: 59 patients with PAD were included in the study. They were 44 males and 15 females with a mean age of 57.96 years. 48 patients (81.35%) were diabetic and 22 (37.3%) were hypertensive.

MTHFR C677T gene polymorphism was analyzed by PCR-RFLP. 85 healthy subjects (36 males and 49 females with mean age of 46 years) served as healthy controls.

Results: The C677T mutation of MTHFR was not found to be different in patients with PAD and controls. 31 patients with PAD (52.54%) and 44 healthy subjects (51.76%) had the wild-type genotype CC, 9 patients (15.25%) and 15 healthy controls (17.65%) had muted TT genotype, and 19 patients (32.20%) and 26 healthy controls (30.59%) had CT heterozygote genotype.

Conclusions: In the PAD population, MTHFR C677T gene polymorphism occurred in a pattern similar to that seen in controls. No significant association was detected between the T/T genotype and PAD.

Abstract number 0053

Implementation of guidelines in patients with very high LDL-C levels in daily clinical practice

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Aim: To evaluate the implementation of lipid guidelines in patients with very high LDL-Cholesterol levels in daily clinical practice.

Methods: A total of 25094 biochemistry records of a tertiary clinic (in and out-patients) during a twelve month period were screened and clinical and laboratory data of patients with very high (≥190mg/dl) LDL-cholesterol levels were analyzed in this observational study. We present the preliminary screening results of the 6464 records during the first 3 month period.

Results: There were 241 patients (3.72% of total, mean age 57.2±11.4, 60.4% male) with high LDL-C levels. Hypertension, diabetes and positive family history were present in 39.1%, 42.6 and 21.6% respectively. Fifty four patients (22.4%) had a diagnosis of any vascular disease. The average LDL-C on first presentation was 217.12±26.7mg/dL (min 190-max 398). Ninety one patients (37.8%) were on statins at initial presentation and an effective dose increase or change to a more potent statin was performed only in 20.9% (19/91). From 150 statin-naive patients 38 (39.25%) were prescribed statins. In the next 12 months a control LDL-C level was available in only 41% (99/241) of patients and mean LDL-C level achieved was 167.6±54.7mg/dL.

Conclusion: Although the importance of LDL-C for primary and secondary prevention is well established this preliminary data of a high risk group suggests that in routine daily clinical practice guideline adherence and follow-up as well as patient compliance is poor.

Abstract number 0054

Hyperhomocysteinemia in patients with coronary artery disease

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Aim: To determine the concentration of plasma homocysteine (Hcy) and the lipid risk factors: total cholesterol (TC), HDL-cholesterol (HDL-C), LDL-cholesterol (LDL-C) and triglycerides (TG) in patients with coronary artery disease (CAD) and healthy subjects, control group, as well as, to investigate the correlation between tHcy and lipid parameters in the set two groups of subjects.
Material and Methods: The investigation included 80 healthy subjects and 80 patients with CAD divided by gender. The concentration of Hcy was determined by the spectrophotometric cyclic enzymatic method, TC and TG and HDL-C were determined by standardized and routine enzymatic methods; LDL-C was calculated by the Friedewald’s formula.

Results: The concentration of Hcy were statistically significant higher in both sex with CAD compared to the control (p<0.001). The levels of lipids were statistically significant higher while HDL-C statistically significant lower in patients in comparison with control group (p<0.05).

There were positive correlations between Hcy and TC, TG and LDL-C, and negative correlation between Hcy and HDL-C in group of men with CAD. Values for X2test (x2=35.48 and p<0.001) have showed a significant association between Hcy concentration and CAD. Increasing the concentration of tHcy for 1μmol/L, is leads to on increased risk for the occurrence of CAD for 25.2%.

Conclusion: The concentration of plasma Hcy is independent risk factor for occurrence and development of CAD.

Abstract number 0122

Paraoxonase 1 polymorphism L55M in patients with primary hypertension – pilot study

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Introduction: Paraoxonase 1 (PON1) is a calcium-dependent esterase associated with the HDL fraction. It is known to protect LDL particles against oxidative modification, leading to formation of atherogenic oxLDL particles, which might be metabolized worse and thus accumulated in blood. The PON1 gene is polymorphic and has been investigated in the development of cardiovascular disease.

Aim: Our study aimed to assess the effect of PON1 L55M polymorphism in patients with primary hypertension and controls.

Methods: We analysed PON1 L55M polymorphism in a total of 17 patients with hypertension and 14 healthy subjects representing both sexes. Fasting blood glucose, lipid profiles, systolic (SBP) and diastolic (DBP) blood pressure and PON1 polymorphism frequency of all of the participants were studied. The PON1 L55M polymorphism was determined by RFLP-PCR (Restriction Fragments Length Polymorphism) analysis.

Results: The following genotype frequencies were determined: 57% LL, 43% LM in control group, 65% LL, 17.5% LM, 17.5% MM in hypertension group. In our study no significant differences were observed in the values of total cholesterol (T-C), LDL cholesterol (LDL-C), HDL cholesterol (HDL-C), triglyceride and fasting glucose between the groups (Mann-Whitney U test). Since PON1 55MM genotype occurred only in hyperten-
sion group we divided all analysed subjects into two new groups: LL (n=19) and LM+MM (n=12). Only in the group carrying M allele, the posi-
tive correlations SBP&T-C (R=0,65) and SBP&LDL-C were observed (R=0,65).

Conclusion: PON1 polymorphism might be responsible for metabolic contribution to arterial hypertension.

Abstract number 0128

Serum oxidized-LDL as an independent risk factor of acute coronary syndrome

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Increased serum oxidized-LDL (OX-LDL) concentration was reported to be an independent risk factor for coronary heart disease. This study was performed to determine levels of the serum OX-LDL and other lipids in patients with acute coronary syndrome (ACS) and to compare them with age- and sex-matched healthy subjects.

The study included 30 patients with ACS (24 with myocardial infarction and 6 with unstable angina) and 20 age- and sex-matched healthy subjects taken as control. All underwent estimation of serum total cholesterol, serum LDL cholesterol, serum HDL cholesterol, serum triglyceride (TG) and serum OX-LDL. The mean age of cases were comparable to controls, with no significant difference (P>0.05). OX-LDL concentrations were measured using a sandwich ELISA method.

The levels of serum OX-LDL in ACS cases were statistically higher than control group (P<0.05). The mean serum OX-LDL value was significantly higher in myocardial infarction group, as compared to unstable angina group (P<0.01). OX-LDL levels showed a graded association with ACS, with increase of ACS incidence with higher OX-LDL values. Serum OX-LDL was significantly raised in ACS patients with high serum LDL (P<0.01) and high serum TG (P<0.01). This means that the effects of serum OX-LDL are magnified in presence of high serum LDL. Increased serum OX-LDL levels in ACS group were not affected by various other risk factors for coronary artery disease, thus high serum OX-LDL is an independent risk factor for ACS.

These data suggest that OX-LDL may be used as a valid and more sensitive marker for evaluating ACS.
Abstract number 0178

Correlation between expression changes of CRP and IL-6 with progression of thoracic aorta aneurysm

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Aortic aneurysm is a degenerative disorder characterized by permanent dilatation of the aortic wall that exceeds the normal diameter by at least 50%. The aim of the work was detection of expression changes of gene CRP and IL-6 from the blood of patients with different stages of progressive TAA, on transcription levels (mRNA) and on serum levels of proteins in comparison with healthy controls.

Blood samples from patients with uncomplicated TAAs (n = 60) were sorted into three groups according aortic diameter. Total RNA isolated from peripheral blood was after reverse transcription amplified by real time PCR using specific primers for CRP and IL-6 in comparing to housekeeping gene expression of Gapdh and Hgprt. Levels of serum CRP and IL-6 were detected using immunoassay ELISA.

Our results showed increasing tendency in mRNA levels for CRP (from 80% in group 1 to 500% in group 3 against control group) followed with similar increase of CRP levels in blood (from 76% in group 1 to 152% in group 3 against control group). On mRNA level there was detected 7,5 times higher expression of IL-6 in group 3 what resulted in the increase of final protein by 84% higher level in group 3 against control group). Further studies on a larger group of patients are needed for better evaluation and confirmation the usefulness of serum CRP and IL-6 as markers of the disease progression.

The work was supported by grant project VEGA 1/0115/14 and KEGA 013 UPJ0160-4/2016

Abstract number 0185

N-glycosylated IGFBP-4 could be less susceptible to PAPP-A mediated proteolysis than the non-glycosylated form

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Background: Pregnancy-associated plasma protein-A (PAPP-A) dependent cleavage of the IGF-binding protein-4 (IGFBP-4) results in the release of active IGF and N- and C-terminal proteolytic fragments (NT- and CT-IGFBP-4). These fragments were recently shown to be strong biomarkers of adverse cardiac events risk in acute coronary syndrome (ACS) patients. Circulating IGFBP-4 is partially glycosylated in the N-terminal part. The impact of this glycosylation on IGFBP-4 proteolysis and the formation of NT- and CT-IGFBP-4 are still unknown. The aim of this study was to investigate the possible influence of glycosylation of IGFBP-4 on the formation of NT- and CT-IGFBP-4.

Methods: Glycosylated and non-glycosylated IGFBP-4 and NT-IGFBP-4 were extracted from plasma samples of ACS patients (n=12) using immunochromatography and concanavalin A chromatography. The proteins were analyzed by mass-spectrometry (MS), Western blotting (WB), and by using specific sandwich immunoassays.

Results: The presence of glycosylated NT-IGFBP-4 (17260 Da) in concanavalin A purified preparation was confirmed by MS. The purified non-glycosylated IGFBP-4 was 3-4 times more susceptible to PAPP-A dependent proteolysis than the glycosylated IGFBP-4. The analysis of individual ACS plasma samples showed that 47.2-61.7% of full size IGFBP-4 was glycosylated, whereas only 9.8-23.5% of the NT-IGFBP-4 was glycosylated.

Conclusion: The PAPP-A dependent proteolysis studies using purified glycosylated and non-glycosylated IGFBP-4 showed that the glycosylation of IGFBP-4 is able to suppress its specific proteolysis by PAPP-A. This is supported by the fact that the amount of glycosylated NT-IGFBP-4 detected in ACS plasma was lower than expected if both forms of IGFBP-4 were to be cleaved with similar efficiency.

Abstract number 0193

Human cardiac troponin T is cleaved by thrombin in serum samples

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Studying the stability of cardiac troponin T (cTnT) in samples of patients with acute myocardial infarction (AMI) we noticed that cTnT is much more stable in plasma samples than it is in serum samples. We suggested that this could be caused by a cleavage of cTnT by coagulation
enzymes, known to be activated in serum. In this work we studied the thrombin-mediated cTnT degradation. The degradation of cTnT was studied using gel electrophoresis, immunoblotting and mass spectrometry analysis (MS). The incubation of recombinant cTnT or native ternary troponin complex in buffer solution containing thrombin resulted in a rapid cleavage of cTnT at the N-terminus and a formation of a 25-kDa product. No cTnT degradation was observed if thrombin was pretreated with hirudin, which inhibits its activity. Similar degradation of cTnT was observed in serum (but not in plasma) samples, collected from AMI patients, as well as in in vitro experiments, when both, recombinant cTnT or ternary troponin complex, were incubated in serum. This cleavage was abolished by preincubation of serum samples with either heparin or hirudin. In addition, no degradation was observed when cTnT was spiked into citrate, EDTA or heparin plasma samples. When the products of thrombin-mediated proteolysis were analyzed by MS, two fragments of 2-68 aar and 69-288 aar were identified. This indicates that thrombin cleaves cTnT between R68 and S69. The results of this study show that cTnT is cleaved in serum by thrombin. This should be considered when studying cTnT degradation or developing new immunodiagnostic systems.

Abstract number 0212

Lipid profile in a patient with familial hyperlipidemia during 18 years of treatment

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Familial hyperlipidemia (FH) is a severe disease characterized by marked hypercholesterolemia and premature coronary heart disease. We reported the lipid profile of a patient with FH during an 18-year period of treatment. Methods: Total cholesterol (TC), low- and high-density lipoprotein cholesterol (LDL-C and HDL-C), triglycerides (TG), apolipoproteins (apoA and apoB) and lipoprotein (a) (Lp(a)) were measured with Cobas analyser, Roche. Results: The male patient was under observation from 1999 at the age 40. His TC and LDL-C were very high (8.1 and 6.0 mmol/l) and TG 2.3 mmol/l. During the observation period he was treated with hypolipidemic drugs combined with LDL apheresis. The level of TC and LDL-C had reduced to near target by the end of 2014 and extended lipid profile revealed near normal levels also for HDL-C, apoA and apoB. Simultaneously measured Lp(a) was 10 times above the reference value. By this time patient had underwent coronary artery bypass surgery and percutaneous coronary interventions for 4 times due to recurrent ACS events. During the last 1,5 years the patient has been regularly receiving apheresis. After each session of the apheresis Lp(a) and LDL-C have decreased 4-6-fold, increasing before the next procedure up to about target level for LDL-C and up to approximately 4-fold above normal level for Lp(a). Conclusion: Despite the fact that LDL-C goal was achieved, the atherosclerotic process continued to progress during the treatment. The most likely cause of advanced atherosclerosis in the patient observed in this study was constantly high Lp(a) level between apheresis procedures.

Abstract number 0225

Reference range for cardiac troponin T measured by hypersensitive assay in physiologic neonates

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Methods for measuring cardiac troponins by hypersensitive assays are widely used in clinical laboratories. However, reference ranges and clinical cut-offs for physiological neonates are missing. The aim of our study was to determine reference range and 99th percentile for cardiac troponin T measured by hypersensitive assay (hs-cTnT).

From 530 newborns 264 who fulfilled inclusion criteria (Apgar score ≥ 7, vaginal labour or section Caesarea, mother without drug abuse or any disease, infection or inborn errors of development, haemolysis of the sample ≤ 1 g/L) were enrolled. Reference range (robust method) for hs-cTnT in umbilical cord blood was 14 (95% CI: 1.1-8.9) to 71 (95% CI: 66.7-75.8) ng/l and 99th percentile was 95 ng/l. Newborns born by vaginal labour had higher values than those born by section Caesarea (p=0.008, 95% CI of difference: 1.2 to 8.1 ng/l). Newborns with haemolysis > 3 g/l had significantly lower values than those with haemolysis ≤ 1 (p<0.001).
Abstract number 0233

Falsely elevated cardiac troponin I results on a fully automatic Beckman Coulter system

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Nowadays diagnostic laboratories often use automated solutions to improve workflow. To achieve a short turnaround time, high acceleration centrifugation and plasma samples are used. We observed a surprisingly high number of elevated troponin I values in routine samples from patients with no clinical symptoms for ACS, especially in the range between our cut-off (30 ng/l) and 500 ng/l.

Implementation of an additional centrifugation was leading to significantly lower results ($p = 0.006$). We tested on 25 routine samples if this was unique for the Access AccuTnI+3 troponin I assay on DxI800 in plasma samples or occurred also in other troponin assays.

We found no significant changes in the cardiac troponin results between the routine on step centrifugation on the Automate800 and extra centrifugation step for the Troponin T hs assay on cobas e411 and the ARCHITECT STAT High Sensitive Troponin-I assay on ARCHITECT i2000SR. However, no significant differences between the two different centrifugation conditions have been found using the Access AccuTnI+3 troponin I on serum samples.

We concluded that the falsely increased troponin I results using the Access AccuTnI+3 occurred most likely due to insoluble fibrin as already previously described by Dimeski et al.. According our results, an additional longer centrifugation time is mandatory when using the Access AccuTnI+3 assay on plasma samples.

Abstract number 0236

Cardiac troponin levels (TnI) in children hospitalized for acute respiratory infections

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Background: One of the most important causes of illness in children in the world are acute respiratory infections; myocarditis can be major complication. Myocarditis is an inflammatory process of the myocardium due to different cause: primarily by the numerous infections agents, autoimmune disease, hypersensitivity reactions and toxins. Miocardial damage can be detected by the use of commercially available troponin assays.

Aim: We want to determinate levels of TnI in children hospitalized for acute respiratory infections on the Pediatric Department General Hospital Kruševac during this winter.

Material and methods: We determine TnI Ultrasensitive in the serum of 37 children hospitalized by acute respiratory infections with some symptoms like a chest pain, fever, palpitations, tachycardia, tachypnea, hypotension, hypovolemia, syncope. We using Advia Centaur XP Immunoassay system, the princip of directly chemiluminescence, using commercial Siemens tests. Childrens age were 13,1 months to 18 years. Normal reference rang for TnI is $<0.07$ng/ ml.

Results: Determinated results were in interval 0.001–21,064ng/ml Elevated levels has only two childrens or 5,34%: 15,6 months old boy – 0,326ng/ml and 18 years old girl – 21,064ng/ml. 35 children,12 girls and 23 boys has concentrations in interval 0,001–0,068ng/ml with mean level 0,008942 $\pm$ 0.052ng/ml.

Conclusion: In our examination group, 94,66% of children in acute respiratory infections have normal TnI levels. TnI is not useful routine screening for myocarditis during acute respiratory infections. But, cardial troponin is a highly sensitive and specific marker of miocardial injury and with other examination like ECG, cardiac ultrasound can help in early diagnosis, monitoring and treatment of patients.

Abstract number 0248

Evaluation of co-peptin, NT-proBNP, pentraxin-3, Lp-PLA2 serum levels in subjects with impaired fasting glucose and impaired glucose tolerance

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Aim: Co-peptin, NT-proBNP, pentraxin-3 and Lp-PLA2 levels have been assessed in serum or plasma in subjects with impaired fasting glucose (IFG), impaired glucose tolerance (IGT) and in healthy control to determine if any could be an early marker for prediabetes.
Material and Methods: Patients were recruited from the endocrinology outpatient department of Hafsa Sultan Hospital, Manisa. Fifty patients were enrolled in each group as IFG, IGT and healthy controls. Co-peptin, pentraxin-3 and Lp-PLA2 levels were determined in venous blood samples by an ELISA method. Plasma NT-proBNP measurement was done by an immunometric chemiluminescence method. For the statistical analyses, ANOVA variance, Banforoni and Pearson Correlation tests were used with SPSS 15.0 program.

Results: The groups were not statistically different in terms of gender, age, height and weight but waist circumferences were statistically different. Co-peptin, pentraxin-3 and NT-proBNP levels were significantly elevated in the prediabetic groups compared to the control group. The correlation analysis in the IGT group revealed that NT-proBNP, pentraxin-3 and co-peptin levels were all positively correlated.

Conclusion: The results of our study support that an inflammatory process is involved and hyperglycemia underneath could have affected tissues and organs in the very early phases of prediabetes. Co-peptin, NT-proBNP and pentraxin-3 levels were significantly different in the prediabetic groups especially in IGT compared to the control group. Therefore, these markers could be potentially used as novel biomarkers in nondiabetic patients for detecting IGT in the early stages.

Abstract number 0251

Impact of lipid markers and C-reactive protein on the value of the 99th percentile upper reference limit for high sensitivity cardiac troponin I

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Objectives: i) to assess the relationship between lipid markers and C-reactive protein (CRP), and high-sensitivity cardiac troponin I (hs-cTnI) in the reference population, and ii) to evaluate the impact of lipid markers and CRP on the 99th percentile upper reference limit (URL) for hs-cTnI.

Methods: 531 questionnaire-identified presumably healthy individuals were enrolled in a single-center, cross-sectional study. Surrogate biomarkers for diabetes, myocardial and renal dysfunction were used to refine the healthy cohort (n=408). Lipid profile, total cholesterol:high-density lipoprotein cholesterol (HDL-C) ratio, non-HDL-C, apolipoprotein AI (apoAI), apolipoprotein B (apoB), apoB:apoAI ratio, lipoprotein(a), small dense low-density lipoprotein cholesterol (LDL-C) and CRP were determined.

Results: Individuals with detectable vs. non-detectable hs-cTnI concentrations more often presented with elevated LDL-C (60% vs. 46%; p=0.002), apoB (73% vs. 61%; p=0.008), apoB:apoAI ratio (53% vs. 40%; p=0.005) and lipoprotein(a) (15% vs. 7%; p=0.015). The apoB:apoAI ratio and to a lesser extent other lipid markers, but not CRP, were positively associated with hs-cTnI concentration in univariate and multivariate analyses. Exclusion of individuals with elevated apoB or apoB:apoAI ratio lowered the 99th percentile URL in the presumably healthy population by 24.0% for both biomarkers (7.5 vs 5.7 ng/L) and in the healthy cohort by 14.5% for apoB (6.2 vs 5.3 ng/L) and by 12.9% for the apoB:apoAI ratio (6.2 vs 5.4 ng/L).

Conclusion: Our study demonstrates that atherogenic lipid markers, particularly apoB or apoB/apoAI ratio, influence the 99th percentile URL for hs-cTnI and highlight the need for a uniform protocol for the selection of the normal reference population.

Abstract number 0255

Prognostic value of routine laboratory tests in patients hospitalized for acutely decompensated chronic heart failure

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Aim of the study: Heart failure (HF) is chronic condition that worsens over time. In acute situations it can be life threatening and requires urgent hospitalization. Despite better treatment during last 20 years, prognosis for these patients is still poor. We analyzed laboratory records of emergency hospitalized patients with acute decompensate chronic HF in order to explore their prognostic value regarding the survival of patients.
Methods: During four months of examination 165 patients were hospitalized for acute decompensate chronic HF. Laboratory tests were done at the beginning, during and at the end of hospitalization. Laboratory results were analyzed regarding the patients survival.

Results: At the end of approximately 9 days of hospitalization 26 of totally 165 patients died (17%). Patients who survive had significantly lower brain natriuretic peptide (NT-proBNP), troponin T (TnT), urea, creatinine and red blood cell distribution width at the beginning of hospitalization than patients who died (P < 0.001). NT-proBNP, TnT, urea and creatinine had good discrimination power between survivors and non-survivors (P < 0.001). NT-proBNP cut-off value was 1254 pmol/L. Patients who survive also had significantly lower uric acid and AST (P < 0.005), lower ALP and GGT and better oxygen saturation (P < 0.05) at the beginning of hospitalization. At the end of hospitalization patients who survived had lower mean platelet volume (P < 0.049) and lower leukocytes (P < 0.004) than patients who died.

Conclusion: Our study demonstrated that NT-proBNP has the best discrimination power between survivals and non-survivals. Routine laboratory tests are immediately available and can help clinicians in assessment of prognosis.

Abstract number 0257

The influence of hypothyroidism and inflammatory processes on serum lipid profile

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Aim: To evaluate atherogenic lipid profile changes as influenced by hypothyroidism and inflammatory processes.

Methods: Thirty patients with clinical symptoms of hypothyroidism were investigated: 15 with overt hypothyroidism and 15 with mild hypothyroidism, among them were 26 women and 4 men and mean age was 44 ± 14 years. The control group of 30 subjects without clinical symptoms of hypothyroidism consisted of 28 women and 2 men and mean age was 45 ± 10 years. Lipid parameters, apolipoprotein B (apo B) and apolipoprotein A I (apo A I), interleukin-6 (IL-6) and C reactive protein (hsCRP) were measured in the serum. Results: Patients with overt hypothyroidism were characterized by atherogenic serum lipid profile. Total cholesterol (TC), LDL cholesterol (LDL-C), non-HDL cholesterol (non HDL C) and triglyceride (TG) concentrations were significantly higher (p < 0.05) than in the control group. In patients with mild hypothyroidism these lipid parameters did not differ significantly from the control group. Concentrations of TC, LDL C, non HDL C and apo B correlated positively with thyroid stimulating hormone (TSH) and inversely with thyroid hormones concentrations (fT3 and fT4) in the whole group of patients with hypothyroidism. IL-6 and hsCRP concentrations were positively correlated with TG and negatively with HDL-C and apo AI. Conclusions: The thyroid hormones deficiency caused the increase in atherogenic lipoproteins concentrations and chronic inflammatory processes lowered anti-atherogenic lipoprotein concentration, so intensification of both processes led to augmented severity changes in serum lipid profile.

Abstract number 0259

The low density lipoprotein oxidation in hypothyroidism

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Aim: To evaluate the relation between LDL oxidation, intensification of inflammatory processes and endothelial damage in hypothyroid patients.

Methods: Thirty patients with clinical symptoms of hypothyroidism were investigated: 15 with overt hypothyroidism and 15 with mild hypothyroidism, among them were 26 women and 4 men and mean age was 44 ± 14 years. The control group of 30 subjects without clinical symptoms of hypothyroidism consisted of 28 women and 2 men and mean age was 45 ± 10 years. Concentrations of oxidized LDL (oxLDL), lipid parameters, apolipoprotein B (apo B) pro-inflammatory markers (interleukin 6; IL-6 and C reactive protein; hsCRP) and endothelial cell markers (sE selectin, sP-selectin, von Willebrand Factor; vWF) were measured in plasma or serum. Results: Patients with overt hypothyroidism had higher concentration of oxLDL (p < 0.05) in relation to control group. However, no difference was found in oxLDL concentration between patients with mild hypothyroidism and control group. The oxLDL concentration correlated positively with thyroid stimulating hormone (TSH) and inversely with thyroid hormones concentrations (fT3 and fT4) in the whole group of patients with hypothyroidism. There was a positive correlation between oxLDL concentration and LDL-C, apo B, TC and TG concentrations. No significant correlation was observed between oxLDL concentration and pro-inflammatory markers (IL-6, hsCRP) and endothelial cell markers (E selectin, P selectin, vWF).

Conclusions: LDL oxidation is directly related to LDL concentration in hypothyroidism and this process was not aggravated by chronic inflammation. The endothelial activation or damage were not modify by augmented LDL oxidation.
Abstract number 0265

The thyroid dysfunction influence on the risk factors of cardiovascular diseases

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Subclinical hypothyroidism (SCH) is a common endocrine disorder characterized by elevated TSH values with normal FT4 and FT3. There is evidence that hypothyroidism is associated with abnormal lipid metabolism, while available data for SCH is rather contradictory. The aim of our study was to assess the relationship between SCH, the lipid profile and inflammatory markers as potential risk factors of cardiovascular diseases, as well as the presence of thyroid antibodies.

Our study included 29 female patients with SCH and 31 female control patients, all aged similarly. TSH, FT4, FT3, anti-TPO and anti-Tg were determined by chemiluminescent immunoassay using UniCel_Dxl600. Analysis of total cholesterol, triglycerides, HDL-cholesterol, LDL-cholesterol, apoA1, apoB and CRP was performed by means of Beckman_CoulterAU480, using reagents from the same manufacturer. CRP, apoA1 and apoB were determined by immunoturbidimetry, while the others were analyzed using enzymatic spectrophotometry tests. Statistical analysis was performed using MedCalc.

T-test analysis yielded statistically significantly higher values of total cholesterol (p = 0.010), LDL-cholesterol (p = 0.008) and apo B lipoprotein (p = 0.008) in patients with SCH. Neither of the tests included in the study correlated with TSH values. Fischer exact test yielded a significantly wider presence of positive anti-TPO in patients with SCH (p = 0.001).

Our results show that abnormal lipid status can be found in patients with SCH. Therefore, a more profound laboratory diagnostics should be performed in order to prevent potential complications associated with CVD. Moreover, the common presence of thyroid antibodies in these patients suggests a higher susceptibility for development of clinical hypothyroidism.

Abstract number 0272

The association of red cell distribution width with renal function after kidney transplantation

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Background: Cardiovascular diseases are the leading causes of death after kidney transplantation (37%). None studies have reported that elevated red cell distribution width is associated with poor outcomes in patients after kidney transplantation. The aim of the study was to assess the factors affecting a red cell distribution width and value estimated glomerular filtration rate.

Methods: The study included 135 renal transplant recipients (3 months in baseline and 12 months after kidney transplantation) (mean age: 51 yrs). Clinical and laboratory data were also analyzed, estimated glomerular filtration rate was calculated with Chronic Kidney Disease Epidemiology Collaboration formula. We compared the results with red cell distribution width as reference calculating percentage of reclassification chronic kidney disease stages.

Results: In 3 month after kidney transplantation mean red cell distribution width was 14.22 ± 0.62 and in 12 months was 13.46 ± 0.48 (P < 0.001). There was a significant correlation between the red cell distribution width in the 12 months after the kidney transplantation and estimated glomerular filtration rate (R = 0.35, P = 0.03). There was not statistically significant correlation between red cell distribution width and hemoglobin (R = 0.03, P = 0.87).

Conclusions: Kidney transplantation significantly reduces the risk of cardiovascular diseases assessed by the red cell distribution width. It was a significant correlation between the red cell distribution width and renal function assessed using the indicator estimated glomerular filtration rate.

Abstract number 0277

Laboratory diagnostics might highlight the metabolic characteristics of patients with obstructive sleep apnea in cardiovascular prevention

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Obstructive sleep apnea is associated with increased risk for cardiovascular disease, however either coexistence of cardiovascular risk factors or cause-effect relationships are discussed.

The aim of the study was to analyze superoxide dismutase-1 activity in erythrocytes of mild and moderate obstructive sleep apnea (OSA) males according to their glucose tolerance results.

Methods: Elevated body mass index (BMI) non-smoking Caucasians aged 30-64 with no acute disease or severe chronic disorder were qualified for the study. OSA-suspected males underwent full-night polysomnography and apnea/hypopnea index (AHI) was used to diagnose mild (AHI 5-15) and moderate (AHI 16-30) OSA. The results of oral glucose tolerance test (G0', G120') allowed to select three groups of OSA males: normal glucose tolerance, NGT (n=28), pre-diabetes, PreDM (n=28) and type 2 diabetes, T2DM (n=28). Fasting plasma lipid profile (T-C, HDL-C, LDL-C, TG) and OGTT results were determined (Dimension Xpand Plus, Siemens Healthcare), and serum insulin (ELISA BioSource, Sunrise Tekan) and erythrocyte Cu,Zn-superoxide dismutase activity, SOD-1 (Randox, StatfaxTM 1904Plus) were measured.

Results: OSA males did not differ in their age and BMI. In mild OSA population some changes of SOD-1 was observed with increasing values in PreDM group, while decreasing SOD-1 from NGT to T2DM group was observed in moderate OSA.

In T2DM group different correlations, like negative SOD-1&AHI, SOD-1&G120' and positive SOD-1&satO2, SOD-1&HDL-C were observed.

Conclusion: Superoxide dismutase-1 activity can fluctuate in the studied groups of males in a context of their glucose intolerance we diagnosed. Decreased SOD-1 in diabetic OSA males might be related to different metabolic factors.

Abstract number 0287

The association of red cell distribution width with cardiovascular risk factors in hemodialysis patients

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Background: Cardiovascular diseases are the leading causes of death in hemodialysis patients (39%). None studies have reported that elevated red cell distribution width is associated with cardiovascular risk factors in hemodialysis patients. The aim of this study was to assess the factors affecting a red cell distribution width.

Methods: The study included 75 hemodialysis patients (mean time of dialysis: 7 yrs). Laboratory data, blood pressure, BMI and waist circumference were also analyzed.

Results: Mean red cell distribution width was 14.94 ± 1.19. There was a significant correlation between red cell distribution width and waist circumference (R = −0.41, P = 0.039), C-reactive protein (R = 0.28, P = 0.042), parathyroid hormone (R = 0.348, P = 0.014), total cholesterol (R = −0.34, P = 0.023), high-density lipoprotein (R = −0.31, P = 0.044). There was not statistically significant correlation between red cell distribution width and time on dialysis (R = −0.05, P = 0.762), age (R = 0.18, P = 0.164), Kt/V (R = −0.14, P = 0.921), ultrafiltration volume (R = −0.187, P = 0.181), body mass index (R = 0.09, P = 0.517), systolic blood pressure (R = 0.011, P = 0.037), diastolic blood pressure (R = −0.05, P = 0.772).

Conclusions: Traditional risk factors of cardiovascular diseases are associated with elevated red cell distribution width. Excessive fat tissue may even have a protective impact on the risk of cardiovascular diseases assessed by RDW—the “obesity paradox”.

Abstract number 0303

Fragmentation of human cardiac troponin I from serum samples of AMI patients

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Blood measurement of cardiac troponin I (cTnI) is one of the most reliable methods of acute myocardial infarction (AMI) diagnosis. It was shown that cTnI in the blood of patients is presented by the intact molecule and a repertoire of proteolytic fragments. Proteolytic degradation may have a significant influence on the precise immunodetection of cTnI. In this study we aimed to border the cTnI fragments present in the blood of AMI patients. Serial blood samples were collected from 9 patients over a period of 5-36 hours following the onset of AMI. cTnI and its
fragments were extracted from blood samples by means of affinity chromatography, studied by Western blotting followed by immunostaining with monoclonal antibodies (mAbs) specific to different cTnI epitopes. A similar set of cTnI forms was found in the blood of different AMI patients. It consisted of the intact cTnI molecule and 11 major fragments with relative molecular mass of 14-24 kDa. mAbs, the epitopes of which lie approximately between 23-158 amino acid residues (aar), stained more than 90% of all detected protein independently of the time when the blood sample was collected. The ratio of different fragments remained constant 5-36 hours after the onset of AMI. We can conclude that the qualitative composition of cTnI fragments in the circulation is constant for 1.5 days following AMI, and almost all detected fragments comprise 23-158 aar of cTnI. Therefore, the region 23-158 aar of cTnI is a preferable target for the antibodies to be used in cTnI immunoassays.

Abstract number 0314

Relationship between cold water swimming and increased cardiac markers? a pilot study

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Drowning causes about half million deaths in the world every year. There is a higher risk of adverse medical events and death from cardiac causes with increasing number of cold water swimming (CWS). Concentration of high sensitivity troponins or natriuretic peptides can be associated with prediction of cardiovascular disease, risk of cardiovascular death, incidental hearth failure, prognosis and mortality.

Eight swimmers (5 men), mean age 44 (31-71) years, were examined during winter swimming competition in November 2014. Six swimmers (4 men) swam 500 m long distance, one woman swam 750 m and one man swam 1000 m long distance. Water temperature was 8.2°C. Concentrations of high sensitivity troponin T (hsTnT), high sensitivity troponin I (hsTnI) and aminoterminal pro-BNP (NT-proBNP) were examined a day before, immediately after, 2 hours after and 24 hours after competition. Trends of hsTnI, hsTnT and NT-proBNP were tested using ANOVA.

Swimming time ranged from 9 minutes and 36 seconds to 26 minutes and 48 seconds depending on distance and velocity. Mean BMI of swimmers was 26.3 (22.1-27.9). There was a statistically significant increase of hsTnI 2 hours after CWS (p = 0.048, quadratic trend). Trends of hsTnT and NT-proBNP did not exhibit statistically significant differences (p = 0.19, p = 0.57 resp.).

CWS can be connected with release of cardiac troponins, however is not connected with release of NT-proBNP. Other research should clarify whether release of cardiac troponins during CWS can be connected with presence of arrhythmias, higher cardiovascular risk or probability of incidental hearth failure.

Abstract number 0329

Best use of red cell distribution width in patients after liver transplantation

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Background: Cardiovascular diseases are the leading causes of death after liver transplantation (LTx). None studies have reported that elevated red cell distribution width (RDW) is associated with cardiovascular (CVD) risk factors assessed by bioimpedance analysis after LTx.

The aim of the study was to assess the factors affecting a red cell distribution width.

Methods: The study included 60 patients after LTx. We use the following research tools: (1) measurement of body composition – the amount of fat in the whole body (FAT%) and abdominal (VISC.FAT%); (2) measurement of the waist circumference (WC); (3) analysis of the past medical history.

Results: Obesity (WC > 80 cm for woman’s and WC > 94 cm for men’s) was demonstrated in 61 % of woman’s and 69 % of men’s. The statistically significant negative correlation was found between RDW and FAT% (R = -0.395; P = 0.002), visceral fat rating (R = -0.312; P = 0.016), visc. FAT% (R = - 0.386; P = 0.003). Statistically significant positive correlation was found between the WC and RDW (R = 0.234; P < 0.032).

Conclusions: Evaluation of red cell distribution width in patients after LTx is a helpful tool in the diagnosis of CVD risk.
Abstract number 0332

Best use of red cell distribution width in patients with chronic kidney disease

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Background: Cardiovascular diseases (CVD) are the leading causes of death in chronic kidney disease (CKD). None studies have reported that elevated red cell distribution width (RDW) is associated with CVD risk factors assessed by bioimpedance analysis in CKD patients.

The aim of the study was to assess the factors affecting a red cell distribution width.

Methods: The study included 80 patients with CKD. We use the following research tools: (1) nutritional status was assessed with a Tanita BC 418 body composition analyzer: the amount of fat in the whole body (FAT%) and abdominal (VISC.FAT%); (2) measurement of the waist circumference (WC); (3) analysis of clinical and laboratory data.

Results: Obesity (defined as WC > 80 cm for woman's and WC > 94 cm for men's) was demonstrated in 57 % of woman's and 58 % of men's. Statistically significant positive correlation was found between RDW and age (R = 0.371; P < 0.001), serum creatinine (R = 0.407; P < 0.001), visceral fat rating (R = 0.215; P = 0.042). The statistically significant negative correlation was found between RDW and eGFR (R = -0.461; P < 0.001). There was not statistically significant correlation between RDW and FAT% (R = 0.027; P = 0.806), visc.FAT% (R = 0.054; P = 0.621), WC (R = 0.115; P = 0.290).

Conclusions: Evaluation of red cell distribution width in group of CKD patients is a helpful tool in the diagnosis of CVD risk factors.

Abstract number 0339

Vascular endothelial growth factor (VEGF) gene polymorphism in coronary artery disease

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Recently, researches focused on effects of genetic factors on the pathogenesis of coronary artery disease (CAD). Polymorphism of angiogenesis regulation genes related predisposition of CAD. Vascular endothelial growth factor (VEGF) plays a very important role in the development of angiogenesis.

We evaluated the association between VEGF -2578 single nucleotide polymorphism and angiogenesis in 123 patients with CAD and 28 healthy controls by PCR method. Serum VEGF, neuregulin, oxidized LDL, nitric oxide and paraoxonase levels were measured using enzyme-linked immunosorbent assays. Total antioxidant capacity levels were spectrophotometrically.

There was not found any significant difference between patients and controls in VEGF gene according to genotype distribution. Also, there was not found any significant difference between groups of patient with diseased vessel according to genotype distribution. However, an increase were observed in serum VEGF, oxidized LDL, nitric oxide ve paraoxonase levels in total patients compared with controls. There was observed significant difference in serum VEGF levels between patient and control groups with CC and CA polymorphism. We found higher oxidized LDL levels in patients with CA and AA polymorphism compared to control group. There were found significantly increased paraoxonase levels in patients with AA polymorphism as compared to controls. The data didn't suggest the significant association between VEGF polymorphism and CAD, however our study indicated that CAD patients have higher VEGF levels than healthy controls, -2578 A/C may be important factor in determining serum VEGF levels.

Abstract number 0361

Normal levels of cardiac troponin I in Kazakh women by a high sensitive assay

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Study aims. This study aimed to determine the 99th percentile upper reference limit (URL) concentration of cardiac troponin I (cTnI) in a cohort of apparently healthy Kazakh women.

Design and methods. Apparently healthy females working in public hospitals were recruited on a voluntary basis and underwent a clinical questionnaire on cardiac risk, laboratory testing for cardiac troponin I by a high sensitive assay (Abbott ARCHITECT hsTnI) and additional assays to exclude preexisting morbidities (BNP, <50 pg/mL; HbA1c, <6%; creatinine, <100 mmol/L).

Results. Of the 518 women originally enrolled, 102 (19.7%) were excluded due to missing data (10), ongoing cardiovascular pathology (74) or bad habits (18) and 67 (12.9%) due to elevated biomarker levels, mainly HbA1c (38). The remaining subjects had a mean age of 35.0 ± 8.6 years with a normal distribution (median: 35; range: 18-80); 97 (27.8%) had >50 years. cTnI was measurable in 60 (17.2%) overall and in 29 (29.9%) of the older group. After eliminating gross outliers (hsTnI >100 ng/L) the 99th percentile was 27.5 among women <50 years, 7.6 in those >50 years and 24.6 overall. The median values increased significantly with age (0.6 vs. 1.0).

Conclusions. The exclusion rate among apparently healthy women was similar to previous studies, mainly due cardio/metabolic factors. Troponin I levels were measurable in a low percentage of cases (22.9% overall and 17.2% of the “true healthy” women). The 99th percentile was surprisingly higher in younger women: a more robust estimate of outliers shall be performed in order to confirm this.
In the present study, 353 type 2 diabetic subjects who were divided according to microalbuminuria and renal status, into two groups: with nephropathy (DNP = 47) without nephropathy (DM = 306) were enrolled. Our study was approved by the institute’s ethics committee. Genotyping for ACE-I/D-rs1799752, ACE-rs4343G>A, AGT-rs5050A>C, AGT-rs4762C>T, AGT-rs699A>G and AGTR1-rs5186A>C was performed by PCR-RFLP. Haplotype and statistical analysis were realized using respectively SNPAnalyzer2.0 and SPSS20.

Genotypes frequencies were in Hardy-Weinberg. After adjustment to potential confounders factors (Age, sex, diabetes duration, hypertension...), an increased risk for DNP was associated with mutated alleles of rs4762 (OR = 11.88, p < 0.001), rs699 (OR = 21.46, p < 0.001), and rs5186 (OR = 12.36, p < 0.001). Whereas, mutated allele of rs1799752 seemed to be protector (OR = 0.38, p = 0.011). Adjusted ORs of DNP associated with the ACE haplotype defined by the rs1799752_D and rs4343_A alleles was (OR = 10.79, p = 0.047) and with ACE-AGT haplotype carrying the rs5050_A, rs4762_T, rs699_A, rs1799752_D, rs4343_A and rs5186_A was (OR = 5.94, p = 0.032).

This study indicates that common variants in ACE, AGT and AGTR1 seem to play a role in genetic susceptibility to diabetic nephropathy in a Tunisian population and provides a first evidence for a disease haplotype: DAATAA (following the upper order).

Abstract number 0171

Crystalluria search assistance type of kidney stones in diabetics

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Introduction: The prevalence of kidney stones in the diabetic population was recently estimated at 21%.

Crystalluria can help identify lithogenic risk factors or metabolic abnormalities, genetic or otherwise, that promote nephrolithiasis, and the lack of calculation to be analyzed, it may point to the nature of it and to particular causes.

The main objective is to clarify the particular type of crystal formation that can be found in diabetics.

Material and method: This is a prospective, descriptive including type 1 diabetes and type 2, crystalluria was examined by optical microscope polarized. These patients underwent a blood and urine assessment, research and identification of possible crystalluria.

Result: This study focused on a population of 72 diabetic patients, divided into 66.67% women and 33.33% men with a sex ratio (M / F) of 0.50, the overall frequency of crystalluria was 69.44% with gender difference. The results of our study show that the average pH of the urine pH was 5.45 ± 0.48 that is to say acid significantly, which promotes dihydrate calcium oxalate crystalluria. Crystalluria also depends on calcium excretion. The dosage of this parameter gives an average of 378.54 ± 127.73 mg/l in men and 362.47 ± 74.97 mg/l in women.

Conclusion: The study of crystalluria is an essential source of information for the etiologic diagnosis and medical management. It should be practiced in all laboratories to enable better detection of risk factors and a more effective monitoring of nephrolithiasis diabetes.

4th Joint EFLM-UEMS Congress

Thursday September 22nd

Posters

Therapeutic drug monitoring and pharmacogenetics of immunosuppresants

Abstract number 0119

The relationship between tacrolimus immunosuppressive treatment and free light chains in patients after heart transplantation

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Background: Patients undergoing solid organ transplantation often exhibit immunoglobulin abnormalities. Tacrolimus affects the regulatory functions of immune system thus influencing both T- and B-lymphocytes.

Objectives: To analyze the effect of tacrolimus treatment on kappa and lambda free light chains (FLC) and their ratio with respect to tacrolimus concentration and area under concentration curve (AUC).
Patients and methods: In a pilot study we evaluated 41 heart transplant (HTX) patients (39 men, 2 women; aged 56-72 years). Blood samples were obtained before, +9, +18 and +24 months post-HTX. FLC and tacrolimus were measured using Binding Site kits on SPA Plus and Abbott Architect kits on Architect ci16200, respectively. Friedman and Kruskall-Wallis tests were used to analyze data.

Results: There was a significant decrease (p < 0.001) in FLC kappa, lambda and kappa/lambda ratio post-HTX compared to pre-HTX values. Patients with higher average TAC concentrations (>11.82 ľg/L) in the first 9 months post-HTX showed significantly lower FLC kappa/lambda ratios in 9th, 18th and 24th month (p = 0.0012; 0.0020; 0.0128, respectively) post-HTX. Similar dynamics were observed for higher (>3579 ľg) tacrolimus cumulative AUC in 9th month. These changes were reflected only in IgG kappa subtype (decreased, 9th month, p < 0.0178).

Conclusion: We found a consistent relationship between tacrolimus concentration and dynamics of FLC kappa, lambda and their ratio. Higher average tacrolimus concentration and cumulative AUC (highest in the first 9 months, with reduction in concentration in the following months) were accompanied by lower FLC kappa/lambda ratio. Therefore, the concentration of tacrolimus can have specific influence on different immunoglobulin subtypes.

Affiliations: The work was supported by the Ministry of Health of the Czech Republic (Grant number: AZV MZ 15-27579A).

Abstract number 0293

Abuse in the use of benzodiazepin derivatives with inversion leucocyte

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From January 2015 and during the following 13 months 56 patients, both sexes and belonging to middle/upper strata of society were studied. They were all undergoing treatment with benzodiazepin derivatives.

They presented macrocytosis, very low white blood cells count, evident thrombocytopenia, altered hepatic profile, anemia in the 95 % of the cases and coagulation tests severely altered, towards hypercoagulability and all had increased leucocytes.

It was decided to suspend the benzodiazepin derivatives. They were helped with acid succinate plus ferrous succinate, and to add prednisone as a way to avoid hepatic disorders, and also a minimum dose of acenocoumarol (adjusted a INR) to prevent prothrombotic effects.

At the next blood control, all the hematological values gradually varied positively so it was decided to suspend the succinate acid plus ferrous succinate and acenocoumarol, but to keep the prednisone until the complete hepatic recovery.

After 90 days, when the corresponding blood control was done, it was shown that the patients had got normal analytical values, with the hemoglobin dosage between 12 and 14,5 g/dl, accompanied with normocytic elements and values of count of white blood cells in normal parameters, thrombocytes between 185 and 395 x 10^9/ml and hepatic dosages with normal reference values and the solution to the inversion of the leucocyte.

The search of psycho-social stability has brought about massive consumption of a large quantity of benzodiazepin derivatives the abuse in the use of these derivatives causes significant quasi-quantitative alterations in the hepatic profile.

Abstract number 0359

Phospho-specific flowcytometry unveils the incomplete blockade of monocyte activation by tacrolimus in kidney transplant recipients

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Aim: To explore the effects of immunosuppression on signaling pathways in monocytes of kidney transplant recipients. Methods: Signaling pathway phosphorylation of NFκB, MAPK (p38, ERK) and AKT was measured with phospho-specific flowcytometry in peripheral blood monocytes of kidney transplant patients (n=20) during the first 6 months after transplantation. Patients received maintenance therapy consisting of tacrolimus, mycophenolate mofetil and prednisolone after receiving basiliximab induction therapy. Both ex vivo phosphorylation and phosphorylation after PMA/Ionomycin stimulation were determined. Individual effects of immunosuppressive drugs on phosphorylation were tested in blood samples from healthy volunteers (n=5). Results: Before transplantation ex vivo phosphorylation of p38MAPK, AKT and ERK, but not of NFκB, was highly expressed by monocytes (MFI: 1684, 1073, 492 and 271, respectively) compared to isotype controls (MFI: 605, 602, 191 and 287, respectively; p < 0.001 for p38MAPK, ERK and AKT). After transplantation phosphorylation was significantly decreased by 23%, 20% and 35% for p38MAPK (figure 1), AKT and ERK, all p < 0.05, respectively. After in vitro stimulation the maximum phosphorylation capacity (MFI: 6902, 2769, 1942, respectively) was 15%, 26% and 5% lower compared to pre-transplant values, all p < 0.05, respectively.
To define the impact of immunosuppressive drugs on p38MAPK phosphorylation, monocytes from healthy controls were studied in either the presence of tacrolimus (50 ng/mL), prednisone (100 ng/mL) or mycophenolic acid (MPA, 10 μg/ml). Only tacrolimus significantly inhibited p38MAPK phosphorylation (p<0.05). Conclusion: Currently prescribed immunosuppressive drugs can inhibit activation of monocyte signaling pathways after transplantation, but the blockade is far from complete allowing for rejection processes to occur under immunosuppression.

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Posters

Laboratory assessment of kidney function

Abstract number 0003

Association of interleukin 18 - 607A/C and - 137C/G polymorphisms with oxidative stress in renal transplant recipients

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Background: IL-18 mediates various inflammatory and oxidative responses including renal injury, fibrosis and graft rejection. It has been reported that the promoter -607 and -137 polymorphisms of IL-18 influence the level of IL-18. This prospective observational study investigated the association between oxidative stress with IL-18 607 and -137 polymorphisms in renal transplant recipients.

Methods: This study included 75 renal transplant recipients (28 female, 47 male) from living related donors. Blood samples were collected immediately before and after transplantation at day 7 and month 1. Serum IL-18, creatinine, cystatin C, CRP and oxidative stress markers (TOS, TAC) were measured. The Oxidative Stress Index (OSI) was calculated. Polymorphisms of the promoter region of the IL-18 gene, IL18-607A/C and -137C/G were determined by analysis of a “real-time PCR/Melting curve”.

Results: Serum creatinine, cystatin C, CRP, IL-18, TOS and OSI levels significantly decreased after transplantation. Posttransplant levels of serum TAC and estimated GFR demonstrated consistent significant increases. Serum IL-18 levels were significantly higher in patients with IL-18-137 GG and IL-18-607 CC genotypes before transplantation. Conclusions: Our results indicate that the IL-18-137 GG and -607 CC genotypes contribute to higher IL-18 levels, however the influence of these polymorphisms on oxidative stress have not been observed.

Abstract number 0030

Category 1 external quality assurance program for serum creatinine

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Objective: To analyze the results obtained by different methods used in Spain to measure serum creatinine, through an external quality assurance program (SCR) with commutable control materials and reference method values.

Methods: 86 Spanish laboratories were involved in this program for a 2015 survey, organized by the Committee of External Programs and the Commission of Analytical Quality of the Spanish Society of Clinical Biochemistry and Molecular Pathology (SEQC) in collaboration with the Dutch Foundation for the Quality. A box with 6 control vials (54 to 262 mmol/L creatinine), stored at - 20 ° C, was sent to the participants. During 6 consecutive days a vial was examined daily for creatinine, measured in duplicate and in a single analytical run.

Results: 990 results of creatinine were obtained. Percentage deviations of the mean values for the 12 methods used respect to the reference value were depicted. Any deviation equal or lower than desirable limit for systematic error derived from biological variation (4%) was considered to be acceptable.

Conclusions: Only the enzymatic methods get all results within the acceptability limits, although it was used by only 6 laboratories. Alkaline picrate kinetic method (compensated or not) show an overestimation of creatinine of 5 to 35% at creatinine concentration lower than 100 mmol/L. Two laboratories were unable to declare their traceability to standards. The lack of standardization of creatinine methods, evidenced in our country, could potentially lead to errors in interpreting laboratory reports.
Abstract number 0050

Association between 1,25-dihydroxyvitamin D3 levels with inorganic pyrophosphate, fetuin A, osteoprotegerin, bone morphogenic protein-2 and alkaline phosphatase in renal transplant patients

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We aimed in our study that examine the relationship between plasma 1,25-dihydroxyvitamin D3 levels with BMP-2 and ALP which activators of vascular calcification, and fetuin-A, OPG and PPi which inhibitors of vascular calcification in renal transplantation patients.

35 renal transplantation patients were included in the research. Assays were performed in the collected blood samples before (PrTx) and after 6 months than operation (PTx). 23 patients were taken lateral lumbar radiograph and made pulse wave velocity (PWV) measurements. By considering the mean age of patients (40.30±12.86 years), 7 m/s was accepted the normal PWV value. Than 2 groups were performed which are PWV < 7 and PWV ≥ 7 m/s.

PTx, that was observed significant increase in plasma 1,25-dihydroksivitamin D3 levels compared to the PrTx (p = 0.0001). There was a significantly increase in serum calcium levels (p = 0.0001) and decrease in serum phosphorus1 with ALP2 levels compared to the PrTx (p1 = 0.001, p2 = 0.011). Increase in Ppi, Fetuin A and BMP-2 levels with decrease in OPG levels were not statistically significant compared to PrTx. 1,25-dihydroksivitamin D3 levels correlated with OPG1 and BMP-22 in both PrTx* and PTx** (r1* = 0.925 ve r2* = 0.762; r1** = 0.574, ve r2** = 0.515). Both period, BMP-2 showed significant correlation with OPG (r = 0.700; r = 0.684).

That can be considered to increase the calcification inhibitors against of increasing calcification risk due to the protection of system as endogenous. In this case, the possibility of vitamin D is calcification inhibitor should not be excluded. It is expected that normalized levels of vitamin D can show a protective effect in CKD patients.

Abstract number 0081

The influence of eGFR calculation method on patients classification to G2 or G3A CKD stage

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The aim of this study was to compare MDRD and CKD – EPI equations for eGFR calculation with respect to patients qualification to early stage (G2,G3A) of chronic kidney disease (CKD).

Data were obtained from 4883 samples referred to the Central Laboratory for serum creatinine. It was measured by standardized Jaffé method and results range was 0,5 – 2,0 mg/dl. eGFR was calculated according to both CKD-EPI and MDRD formulas.

Results and conclusions: In the whole population, for both sexes, number of patients with eGFR CKD-EPI below 60ml/min was smaller than with MDRD method (52% vs 59% for men, 38% vs 42% for women) what might suggest higher diagnostic sensitivity of MDRD, but applies only to situation based on cut off point of 60 ml/min. In higher eGFR (60-70 ml/min) and in G2 (60-89 ml/min) and G3A (45-59 ml/min) eGFR did not differ significantly: G2 EPI 32% vs 34% for men, EPI and MDRD 28% for women, G3A EPI and MDRD 22 % for men, 19% vs 21% for women, so it can be stated that the performance of eGFR calculated by CKD-EPI and MDRD formulas is very similar, in the early stage of chronic kidney disease (G2, G3A) and has no influence on interpretation of estimated values and patients reclassification into other stage of renal disease.

Both eGFR showed gradual decreasing in each decade of age (20-80 years).

Abstract number 0082

The correlation of urinary beta-trace protein with severity of albuminuria in renal disease

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Albuminuria is an important measure for screening, diagnosing, defining severity of kidney dysfunction, estimating prognosis of outcome and guiding therapy treatment in chronic kidney disease (CKD). Beta-trace protein (BTP) has been shown that might serve as an alternative
biomarker of renal injury, especially in the early stages. The aim of this study was to investigate the correlation of urinary BTP with severity of albuminuria in patients with CKD.

The study included 106 patients with CKD, who were divided into 3 groups according to urinary albumin concentration: normal, microalbuminuria and albuminuria. The 24-hour and second morning urine samples were collected from each patient. Urinary BTP and albumin were measured by immunonephelometry (BNII, Siemens).

The comparison analysis (Mann-Whitney U-test) showed significant increase of BTP levels with magnitude of albuminuria. For 24-hour urine samples median values were: 2.12, 4.73 and 14.36 mg/day, and for second morning urine samples: 0.198, 0.465 and 1.670 mg/mmol creatinine, in different albuminuria groups. The data from ROC analysis showed that urinary BTP has a high diagnostic value for detection of > 30 mg/day albuminuria; AUCs were 0.871 and 0.827 for 24-hour and second morning urine samples, respectively.

The results of the study showed good correlation of urinary BTP levels with the magnitude of renal injury (defined by albuminuria), regardless of the type of urine sample.

Abstract number 0208

Plasma neutrophil gelatinase-associated lipocalin (NGAL) can be a useful tool to predict deleted graft function (DGF) after kidney transplantation

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Introduction: NGAL can be released by renal tubular cells in response to inflammatory or injury. It is considered as a biomarker for prediction of graft dysfunction after kidney transplantation (KTx).

The aim of the study was an assessment of two variables: plasma concentration of NGAL-1 and NGAL-2 as a significant predictive factor for confirmation or not a DGF appear after KTx.

Materials and methods: Study population comprised of 56 kidney recipients. NGAL plasma test was performed (The NGAL Test: BioPorto Diagnostics on Roche Cobas 6000) before KTx and repeated on the first (NGAL-1) and the second (NGAL-2) day after. We also assessed number of DGF.

Results: Results of ANOVA analysis and nonparametric version of ANOVA test (Welch) for the model of NGAL-1 and for the model of NGAL-2 showed that both models can significantly predict DGF (p<0.05). Logistic regression was conducted for both models and it has shown that NGAL-1 and NGAL-2 significantly help to predict whether or not a DGF will appear. Furthermore our study suggests that the odds of estimating correctly DGF improve by 6% (p=0.002) if one knows NGAL-1 and 7% (p=0.003) if one knows NGAL-2. When both variables are considered together (NGAL-1 and NGAL-2) they significantly help to predict whether or not DGF appear and the odds of estimating correctly DGF improve by 3% if one knows NGAL-1 and by about 5% if one knows NGAL-2.

Conclusions: NGAL concentration measured on the first and/or at the second day after kidney transplantation can be a useful tool to predict (DGF).

Abstract number 0250

Estimation of serum creatinine enzymatic UV method (Randox) at three different concentration levels

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The goal of the study was to determine the total error of serum creatinine measurement by enzymatic UV method with creatinine deiminase (Randox) in three different concentration levels and assess whether it meets the desirable analytical criteria. Low linearity limit of 20 µmol/L has shown to be a limiting factor for the pediatric samples measurement.

Calibrator Beckman Coulter - REF 66300 was used to calibrate the method and the calibrator C.f.a.s. Roche - REF 10759350 was used as the material to measure accuracy and imprecision. This calibrator is diluted with charcoal striped serum in order to produce samples with final serum creatinine concentration of 338 µmol/L, 48.3 µmol/L and 33.8 µmol/L. The samples were measured in triplicate for five days on the biochemistry analyzer AU680 (Beckman Coulter).
Method: We evaluated 102 consecutive, newly diagnosed patients within a hospital-based cohort. Morning hours spot urine samples were collected before HAART, and 1, 3, 6, and 9 months of treatment. They were treated with Tenofovir disoproxil fumarate (TDF)-emtricitabine with efavirenz (n = 33), zidovudine-lamivudine with nevirapine (n = 33), and zidovudine-lamivudine with efavirenz (n = 53), and zidovudine-lamivudine with efavirenz (n = 53). Diabetes mellitus and hypertension were excluded. Urine samples were processed and examined for crystals microscopically.

Aim: To determine the effects of HAART on crystalluria in previously antiretroviral-naive HIV-infected subjects.

Results: Urine crystals showed the following pattern: Calcium oxalate crystals at baseline: nil in 20% and ++ in 25.7%; reduced at 9 months on HAART to: nil in 4.4% and ++ in 20%, +++ in only 8.6%. Sulphonamide crystals at baseline: nil in 95.7%, + in 1.4%, +++ in 2.9%; with marked improvement after nine months on HAART, viz: 100% nil. Sulphathiazole crystals at baseline: nil in 92.9%, + in 1.4%, ++ in 1.4% and +++ in 4.3%; also improved after treatment: nil in 97.1%, + in 1.4% and ++ in 1.4%. Uric acid crystals at baseline: nil in 98.6% of the patients, +++ in 1.4%; had minimal reduction at nine months, i.e nil in 98.6% and declined to ++ in 1.4%.

Conclusion: HIV infection causes renal parenchymal damage that results in crystalluria. Thus, crystal-induced nephropathy can occur. Calcium oxalate crystals are the most prevalent in treatment-naive persons. HAART markedly improves all forms of crystalluria and could resolve sulphonamide crystalluria.

Abstract number 0264

Preventive noninvasive examination of the renal function in 13-19 years old teens in correlation with physical activity

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Introduction: Continuous preventive monitoring of the adaptation response of the renal system in correlation with physical effort is crucial for identifying of inborn or acquired diseases that might lead to irreversible lesions.

Aim. The purpose of our investigation was to evaluate the results obtained from the preventive noninvasive examinations of renal function of 13-19 years old examinees actively involved in the training and those not actively included in the training process.

Methods: Our study included 480 male examinees in experimental group (active sportsmen in tennis, basketball and volleyball clubs) and 410 healthy male teens in control group, recruited from primary and secondary schools, nonsportsmen. All examinees from the experimental group should have been actively involved in the training process for more than six months. For noninvasive evaluation of the renal function were examined quantitative and qualitative content of urinary proteins with Meulemans' spectrophotometric method and horizontal 4-22% gradient gel SDS-PAG electrophoresis, using samples of second morning urine and urine excreted after physical effort.

Results: The functional proteinuria (orthostatic or sport proteinuria) were detected in 2.9% and 2.2% of examinees from the experimental group and in 3.7% of examinees from the control group. There was a significant difference in excretion of total proteins and in SDS-PAGE profiles between individuals with and without orthostatic and sport proteinuria.

Conclusions: The preventive noninvasive examinations of the renal function are useful for ontime identification of teens with increased risk for developing of pathological response to increased physical effort and irreversible damage of organic systems.

Abstract number 0271

Effects of highly active antiretroviral therapy (HAART) on crystalluria in previously antiretroviral-naive HIV-infected patients

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Aim: To determine the effects of HAART on crystalluria in previously antiretroviral-naive HIV-infected subjects.

Method: We evaluated 102 consecutive, newly diagnosed patients within a hospital-based cohort. Morning hours spot urine samples were collected before HAART, and 1, 3, 6, and 9 months of treatment. They were treated with Tenofovir disoproxil fumarate (TDF)-emtricitabine with efavirenz (n = 33), zidovudine-lamivudine with nevirapine (n = 33), and zidovudine-lamivudine with efavirenz (n = 53). Diabetes mellitus and hypertension were excluded. Urine samples were processed and examined for crystals microscopically.

Results: Calcium oxalate crystals at baseline: nil in 27.1% of patients, + in 14.3%, ++ in 32.9%, +++ in 25.7%; reduced at 9 months on HAART to: nil in 67% of patients, + in 4.4%, ++ in 20%, +++ in only 8.6%. Sulphonamide crystals at baseline: nil in 95.7%, + in 1.4%, +++ in 2.9%; with marked improvement after nine months on HAART, viz: 100% nil. Sulphathiazole crystals at baseline: nil in 92.9%, + in 1.4%, ++ in 1.4% and +++ in 4.3%; also improved after treatment: nil in 97.1%, + in 1.4% and ++ in 1.4%. Uric acid crystals at baseline: nil in 98.6% of the patients, +++ in 1.4%; had minimal reduction at nine months, i.e nil in 98.6% and declined to ++ in 1.4%.

Conclusion: HIV infection causes renal parenchymal damage that results in crystalluria. Thus, crystal-induced nephropathy can occur. Calcium oxalate crystals are the most prevalent in treatment-naive persons. HAART markedly improves all forms of crystalluria and could resolve sulphonamide crystalluria.
Abstract number 0281

Is cystatin C a dialysis adequacy marker?

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Low flux (LF) hemodialysis allows to remove small molecules such as urea and creatinine. High flux (HF) hemodialysis may give possibility of remove middle molecules like cystatin C. There are few studies on changes in cystatin C levels during hemodialysis. The aim of study was to evaluate the efficacy of hemodialysis using reduction ratio for cystatin C during LF and HF hemodialysis.

Material and methods: 55 patients were hemodialized using LF dialyzers (F6HPS n=5, F7HBS n=11, F8HPS n=12, F10HPS n=6, Pf17L n=23) and 10 patients using HF dialyzers (F80S n=6, Pf170H n=4). Dialyzers Hemoflow F were produced by Fresenius Medical Care and Polyflux were produced by Gambro. Cystatin C was measured by immunoturbidimetry on CMD 800iX1 Wiener lab Group. The formula for the calculation of reduction ratio for cystatin C: RR(%) = Co-Ci/ Co x 100%, Co = pre-dialysis cystatin C level, Ci = post-dialysis cystatin C level.

Results and conclusion: Cystatin C was increased after LF hemodialysis with the exception of Pf17L dialyzer and decreased after HF hemodialysis. Difference between pre- and post-dialysis cystatin C level was only statistically significant with F8HPS (6,47 ± 1,27mg/l versus 7,18 ± 1,54mg/l, p<0,05). But the difference in the mean values of cystatin C reduction ratio between the LF (-3,50 ± 10,78%) and the HF (8,23 ± 28,825%) hemodialysis is statistically significant (p<0,05). In conclusion: cystatin C cannot be used as dialysis adequacy marker.

Abstract number 0288

Performance of the EGFR formulas MDRD and CKD-EPI in relation to the blood pressure in renal transplant recipients

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Background: Monitoring of the function of the transplanted kidney is one of the superior elements of adequate therapeutic actions. The aim of the study was a comparative assessment of values of estimated glomerular filtration rate (eGFR) with the Modification of Diet in Renal Disease (MDRD) and the new equation Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) among the renal transplant recipients.

Methods: The study included 100 renal transplant recipients (mean age: 55 yrs, mean time from kidney transplantation 6 yrs). Clinical and laboratory data were also analyzed, eGFR was calculated with MDRD and CKD-EPI formula. We compared the results with MDRD as reference calculating percentage of reclassification chronic kidney disease (CKD) stages.

Results: In systolic hypertension group of patients mean eGFR calculated with MDRD formula was 51.93 ± 19.92 mL/min/1.73m2, and CKD-EPI formula was 55.89 ± 22.05 mL/min/1.73m2, P<0.001. In diastolic hypertension group mean eGFR calculated with MDRD formula was 47.39 ± 21.62 mL/min/1.73m2, and CKD-EPI formula was 51.14 ± 24.30 mL/min/1.73m2, P<0.001. There was no correlation with systolic and diastolic blood pressure and eGFR both calculated with MDRD and CKD-EPI formula (R<0.05, P=NS).

Conclusions: Our data indicates that: (1) with systolic hypertension MDRD formula shows values which are on average 7% lower than in the CKD-EPI; (2) there was no association with values of eGFR and blood pressure after kidney transplantation.

Abstract number 0294

Delta check for creatinine at screening of chronic kidney disease

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The materials and methods: We observed 147 patients who were undergoing planned maintenance twice a year in our multidisciplinary clinical diagnostic laboratory. One of the determined analytes was creatinine (Jaffe) with the obligatory calculation (the equation CKD-EPI) of glomerular filtration rate (GFR).

The results and discussion: According to Russian national guidelines “Chronic kidney disease (CKD): the basic principles of screening, diagnosis, prevention and treatment approaches (2012)” if GFR <60-89 ml/min/1.73 m2, the CKD diagnosis can be established only by laboratory examination when criteria “resistance” are proven. Therefore, the problem of the reliability of changes in the concentration of serum creatinine is of particular importance.
The reference change value (RCV) was 15.2 µmol/l (SD = 2.02; SDi = 5.09 µmol/l) for being used in the laboratory analytical system (AU400 (Beckman Coulter)). In the primary investigation 14 people with GFR < 90 ml/min were identified. Three of them had GFR < 60 ml/min. The results of serum creatinine and GFR changes during the re-investigations had been compared with RCV for determining the changes’ reliability.

According to the data obtained for 9 patients CKD (stage 2) was confirmed. These patients require additional tests for treatment selection. The suspicion in CKD (stage 2) of three patients was not confirmed (convalescence). Two patient’s situation can be considered as uncertain and require an in-depth examination. Thus, despite the fact that in most cases the level of serum creatinine was within the reference interval, using Delta check allowed to prove the development of CKD risks.

**4th Joint EFLM-UEMS Congress**

**Thursday September 22nd**

**Posters**

**Trends in pediatric laboratory medicine**

**Abstract number 0035**

**Serum vitamin D and alkaline phosphatase: seasonality and status during growth**

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Introduction: Lack of sun exposure is one of the primary causes of 25-hydroxyvitamin D (vitD) deficiency and carries a loss of mineral bone density (MBD). In pediatrics, MBD loss is correlated with an increase in alkaline phosphatase (ALP). In this study, status of parathyroid hormone (PTH), ALP and vitD were assessed, along with their relationship in the bone-growing population (<22 years) according to seasonality.

Methods: A one-year retrospective analysis was performed. Inclusion criteria: a complete biochemical analysis including ALP, 25-hydroxyvitamin D (Architect, Abbott) and iPTH (Cobas, Roche). Patients with any illness or chronic treatment found in the hospital records were excluded. Data were analyzed according to semester seasonality: spring-summer (SS) vs. autumn-winter (AW). Variables were compared using the Mann-Whitney’s U-test and Spearman’s Rho correlations, statistical significance was set at 5%.

Results: A total of 263 patients were included: 184 in AW (58% male; median: 14y; p5-p95: 1-21y) and 83 in SS (55% male; median: 10y; p5-p95: 1-21y), p = 0.004. Median vitamin D levels were 26ng/mL (AW) and 30ng/mL (SS), p = 0.004. Median ALP levels were 196U/L (AW) and 221U/L (SS), p = 0.035. No differences were detected in PTH concentrations (p = 0.63). Vitamin D correlated with ALP activity (r = 0.3; p = 0.003), but none showed a significant correlation with PTH. In a subgroup analysis, vitamin D and ALP correlated in the AW group (r = 0.41; p = 0.002), but not in the SS.

Conclusions: In growing children, lack of sun exposure in autumn and winter is related with reduced vitamin D concentration, which levels positively correlate with ALP as biomarker of bone metabolism. However, time of analysis may have a meaningful impact on ALP activity, hence existing significant differences in children before and after puberty.

**Abstract number 0088**

**Usefulness of Apt-Downey test in neonatal haematemesis at emergency laboratory**

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Introduction: Swallowing maternal blood at the time of delivery or cracks on the nipple during breastfeeding are the most common causes of suspected gastrointestinal bleeding in the neonate. The Apt-Downey test is a useful tool in the diagnosis.

Objective: To illustrate the consequences of a dubious Apt-test.

Methods: We present a newborn admitted stable at the maternity unit. Within the first 48 hours he presented an episode of haematemesis with abundant fresh blood in the aspirate. His mother showed an important right nipple crack. It was decided to perform Apt-test to confirm the presence of neonatal bleeding. Neonatal and adult blood was used as positive and negative controls.
Results: Sample color offered doubts about the possible existence of fetal hemoglobin (HbF). Blood test showed mild anemia (hemoglobin: 9.3 g/dL) and unchanged coagulation in the newborn. There’s no evidence to abruptio placentae nor fetal-maternal transfusion. Possible existence of HbF in maternal blood was studied by HPLC without objectified presence. A new bloody aspirated sample was received for Apt-test and which had a negative result. It was concluded that the haematemesis was secondary to swallowed maternal blood. The patient was discharged 7 days after admission.

Conclusions: The presence of hemorrhagic disease in the newborn is rare and should be suspected by coagulation disorders. The Apt-Downey test should be used as screening for gastrointestinal bleeding in the Emergency Laboratory. However, we recommend the implementation of chromatographic techniques for diagnostic confirmation, to avoid unnecessary diagnostic tests from inconclusive Apt-test results.

Abstract number 0133

Complementary proteins: ceruloplasmin, lactoferrin and myeloperoxidase in newborns meconium

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Background and aims: Ceruloplasmin (CP), Lactoferrin (LF), Myeloperoxidase (MPO) constitute the complementary panel modulating oxidative stress. The aim of the study was to determine the concentrations of three metal-containing proteins in meconium and demonstrate the interrelationships between them in the environment of intrauterine fetal development.

Methods: The proteins concentrations were determined by ELISA Kits. All subsequent meconium samples (n=80) were collected from each healthy full-term newborns (n=19).

Results: The CP, LF, MPO concentrations in a single meconium portion [μg/g] were: mean ± SD: 312.4 ± 229.7 (range 52.2-1076), 45.6 ± 78.9 (range 1.7-511.4), 1.8 ± 1.7 (range 0.02-8.8), respectively, while the amounts accumulated proteins in meconium of one newborn [mg] were: mean ± SD: 5.5 ± 6.6 (range 1.1-177.7), 0.8 ± 0.7 (range 0.05-2.7), 0.021 ± 0.015 (range 0.006-0.066), respectively. Statistically significant correlation were between LF vs. MPO in single samples (R=0.354, p=0.0013) and between CP vs. LF in single samples and accumulated proteins (R=0.459, p=0.000019 and R=0.544, p=0.016, respectively). The correlations in the ranges: 0-25%, 25-50%, 50-75%, 75-100% of the total weight of meconium were statistically significant in the range 0-25% of the total weight of meconium between CP vs. LF, CP vs. MPO, LF vs MPO (R=0.782, p=0.0002, R=0.49, p=0.046, R=0.505, p=0.038, respectively).

Conclusions: Three metal-containing proteins present in the meconium, can be involved in the processes of oxidation-reduction in the period of intrauterine. The strongest correlation between CP vs. LF proves their participation in the metabolism of iron. This can help to maintain homeostasis especially in the initial period of intrauterine fetal development.

Abstract number 0159

Implementation of a non-invasive foetal RHE genotyping test for the monitoring of anti-RH3 immunised pregnant women

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Background: Foeto-maternal blood groups incompatibilities are responsible for the haemolytic disease of the foetus and new-born (HDN). This disease is caused by maternal antibodies targeting the foetus red blood cells which leads to anaemia and hyperbilirubinemia. Anti-RH3-immunised women are monitored clinically and biologically towards the end of their pregnancy and after delivery to prevent the consequences of the HDN.

Aim: A non-invasive foetal RHE genotyping test has been developed to screen RH3 foeto-maternal incompatibilities requiring heavy clinical and biological monitoring.

Methods: DNA from 37 plasma of RH:3 women between 12 and 38 weeks amenorrhea were isolated using both manual and automatic methods. The RHE allele was detected by PCR using an adapted published method (Finning et al. Transfusion, 2007, 47: 2126-33). We then compared the genotype result with the RH3 phenotype of the newborn.
Results: With automatic extraction, 34 plasmas were analysed. Results showed 2 false negatives and no false positive (Se = 87.5%, Sp = 100%). With manual extraction, 36 plasmas were analysed. Results showed 2 false negatives and 1 false positive (Se = 87.5%, Sp = 95%).

Conclusion: With manual DNA isolation, a conclusion must be drawn with 2 separate tests. When using an automated method, those two tests are only required to confirm a negative result. Any negative result is confirmed on another sample, later during pregnancy. Non invasive foetal RHE genotyping will be used to screen only the foeto-maternal RH3 incompatibility pregnancies requiring clinical and biological monitoring.

Abstract number 0165

Does inflammatory bowel disease and food allergy coexist in children?

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Introduction: One of the symptoms of inflammatory bowel disease (IBD) is failure of gut barrier, what may allow absorption of various products from the intestinal lumen. Components of food may activate the host immune system and lead to recruitment of inflammatory cells and development of food allergy. Confirmation of allergy and identify of irritating products may provide a bias for the selection of appropriate elimination diet to complement pharmacological therapy.

Methods: In study group included 96 children aged 3-18 years (14 ± 3): 46 children with ulcerative colitis (UC) and 50 with Crohn’s disease (CD). All children were taken blood samples for total IgE and specific IgE to food allergens (egg white, milk, fish, wheat, peanut, soybean) which were determined with ImmunoCAP assays on Phadia 100.

Results: For the whole group, total IgE concentration was 101 ± 112 kIU/L (2.00-961), wheres in children with UC was 115 ± 134 kIU/L (2.00-961) and in those with CD was 87 ± 91.1 kIU/L (2.00-715). In 6 cases: 3 patient with UC and 3 with CD (6.25%) we observed the presence of specific IgE to food allergens. In serum of these patients we observed highly elevated concentration of IgE.

Discussion: At the Falk Symposium No 179, 2011 we have shown that total IgE in serum was increased only in a small part of the IDB children. These results have been confirmed by us in numerous group. Coexistence of allergy with IBD requires further study. It appears advisable to verification of IgG-dependant reactions, involved results in more delayed responses compared to IgE-dependant allergy.

Abstract number 0211

Evaluation of urinary proteins in children with febrile conditions of non-renal origin

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The study presents the results from biochemical evaluation of urinary proteins in second morning urinary samples excreted from children with febrile condition of non-renal origin during the increased body temperature. Urinary samples of 30 children 2-7 years old with febrile conditions were analysed (17 with viral infection of upper respiratory tract, 4 with Coxitis, 6 with Angina lacunaris and 3 with Tonsillopharingitis acuta). Control group comprised 24 age-matched healthy children. Concentrations of total urinary proteins (TP), P/C ratio and the activity of the urinary enzyme NAG were determined using spectrophotometric methods. Electrophoretic separation of urinary proteins was performed by 4-22% gradient SDS-PAGE, using Coomassie Blue R-250 staining technique. There were pronounced differences in concentrations of TP, P/C ratio and NAG activity between subjects with febrile conditions and controls (p < 0.002 for TP, p < 0.001 for P/C ratio and p < 0.001 for NAG). In the examinees with febrile conditions of non-renal origin a characteristic electrophoretic profile was noticed with apparent albumin fraction and apparent fractions of low molecular proteins, which returned to normal like in healthy children, after administration of the antipyretic and antibiotic therapy and decrease of body temperature, with only weak albumin fraction present. Only in one of the children with upper respiratory tract viral infection, proteinuria of mixed glomerular and tubular origin was detected. The results have shown that this approach is appropriate for rapid distinction between functional proteinuria in febrile conditions of non-renal origin from proteinurias connected with renal or systemic disease.
Abstract number 0217

Is it reasonable to test asymptomatic parents and siblings of children clinically suspicious for alpha-1-antitrypsin deficiency?-single center pilot experience

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Background and aim: Alpha-1-antitrypsin deficiency (AATD), a genetic condition associated with lung and liver disease, often remains unrecognized. For this reason preventive measures' success is highly limited. Study aimed to evaluate whether concomitant AATD testing of asymptomatic parents and siblings together with children referred for testing due to AATD clinical manifestation may improve detection efficiency.

Methods: In total AATD was tested in 23 subjects: seven unrelated children clinically suspicious to have AATD (two girls and five boys, aged 2-18 months), both parents of each and siblings of two probands. AATD testing included AAT immunonephelometric quantification and PCR-reverse allele specific hybridization assay for detection of Z and S deficiency alleles.

Results: AATD was confirmed in three clinically suspicious children, all having ZZ genotype and AAT concentration significantly below reference range. All probands' parents were heterozygous carriers of Z allele, although in two cases AAT level was in the reference range. Brother of one of the children with AATD was detected as a heterozygous carrier. Another three probands were heterozygous carriers of Z allele. The same genotype was detected in one of their parents, father in one and mother in two cases, accompanied with AAT concentration fitting the reference range in one case. In one case and his parents no AATD was detected.

Conclusion: Identification of the carrier state in more than half of the asymptomatic persons provides pilot evidence to consider the tested approach as rational. Nevertheless, it should be confirmed in larger, prospective studies.

Abstract number 0286

Pseudohyperkalemia in capillary whole blood samples – an accidental error or a repeatable problem in pediatric hospital?

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Capillary whole blood sample is widely collected for acid-base balance (ABG) and ions concentration evaluation in pediatric individuals. Potassium is one of the most frequently tested analyte. The aim of the study was to analyze frequency of increased potassium in capillary whole blood samples and if it is verified by serum ions analysis.

A retrospective analysis of capillary whole blood samples (ABG with ions concentration) from 3280 pediatric patients, aged 0-18 years, was made. Analysis of samples was performed with Radiometer ABL 90 Flex. Hyperkalemia was defined as increase of potassium concentration over reference ranges.

Pseudohyperkalemia was suspected in 681 samples (20.8% of all analyzed samples), including 72 newborns, 150 infants and 459 children aged 1-18 years. Measurement of ions concentration in serum was repeated in 622 (91.3%) cases, while another test in capillary blood was performed in 69 (10.1%) cases. Mean capillary blood potassium concentration in samples suspected of hyperkalemia was 6.22 ± 1.04 mmol/L, whereas serum potassium concentration was 4.66 ± 0.67 mmol/L, p<0.0001. The highest values were obtained in Newborn Ward (up to 12.3 mmol/L) and the highest mean K+ value was found in specimens collected in Surgical Ward (7.12 ± 1.64 mmol/L). Hyperkalemia in serum sample was confirmed for 55 (8.1%) patients, the values were 6.52 ± 1.16 mmol/L - whole blood vs 5.84±0.68 mmol/L - serum. In 9(1.3%) samples, despite hyperkalemia in whole blood samples (6.28 ± 0.75 mmol/L), hypokalemia in serum (3.1 ± 0.34 mmol/L) was detected, p=0.0039.

The frequency of hyperkalemia in capillary whole blood samples is high and may mask potassium homeostasis disorders.
Abstract number 0318

Is vitamin D deficient in children under 2 years old in our area?

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Background: Vitamin D is known for its role in promoting skeletal health, and recent years studies show that optimal levels of vitamin D protect against autoimmune diseases, cancer, mental balance and ensure the preservation of memory.

The aim of this study was to analyze the level of 25 hydroxyvitamin D in children under 2 years old, admitted in Children’s Hospital Sibiu with various pathologies.

Method: We studied 100 children, aged between 1 month - 2 years old,. The tests have been done between 15 August-15 November 2015,. The laboratory determined the 25(OH) vitamin D level, using ELFA method, Vidas PC analyzer, BioMerieux. The optimal level is considered to be ≥ 30 ng/ml.

Results: 55.5% of all tested children, aged between 1 month - 2 years old, had levels of 25 (OH) vitamin D below 30 ng/ml, with the group media of 28.68 ng/ml (SD 4.2) and the deficiency group media of 22.61 ng/ml (SD 4.1).

Conclusions: Determination of 25(OH) vit D is an important test for children below 2 years old, in order to settle an efficient rickets prevention at this age.
Abstract number 0067

Immunophenotyping in multiple myeloma

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Multiparametric flow cytometry (MFC) has become an invaluable tool in the management of multiple myeloma (MM). MM is characterized by expansion of clonal plasma cells (PCs), which are characterized by loss of markers of mature B lymphocytes (surface CD19, CD45, HLA-DR, Ig) and PCs acquisition markers (surface CD38, CD56, CD138, intracytoplasmic Ig). MFC allows to screen large number of events and to detect multiple antigens at the same time.

The study population consisted of 230 patients with a previous diagnosis of MM. MM was diagnosed in 76 cases (33%), 53 of them - women, 23 - men with a median age of 66 years (range, 45-89 years).

MFC was performed using monoclonal antibodies against CD56, CD19, CD138, CD45 and IgG, IgA. Our analysis used CD138 and CD45 gating for PCs. Monoclonality was confirmed by immunoglobulin light chain analysis (λ, κ). It is known that normal PCs phenotype has CD138+CD45+/lowCD19-CD56-. Using this combination of monoclonal antibodies allowed us to identify the following variants of the myeloma cell antigenic profile:

1. CD45-CD138+CD19-CD56+ (51%, n = 39)
2. CD45+CD138+CD19-CD56+ (21%, n = 16)
3. CD45-CD138+CD19-CD56- (12%, n = 9)
4. CD45+CD138+CD19+CD56- (4%, n = 3)
5. CD45+CD138+CD19-CD56+/CD19-CD56- (12%, n = 9)

The positive expression rates of IgG, IgA and immunoglobulin light chain κ, λ in neoplastic myeloma cells were 57%, 43%, 63% and 37%, respectively.

Although MFC allows not only for identification and characterization of neoplastic PCs, this approach is used in routine diagnostics of MM. In addition, immunophenotyping is an indispensable method in prognostic classification and management of MM.

Abstract number 0167

Evaluation of new prognostic markers for detection of endometriosis

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Endometriosis is an estrogen-dependent disease with prevalence around 6-10%. One from the suitable markers for detection of endometriosis is the beta-catenin, which affects the regulation of transcription of genes involved in the Wnt signaling pathway and cell adhesion. Another suitable marker for screening of endometriosis is the HIF1 (regulated by the nuclear factor κB).

The aim of the work was detection of changes in gene expression of beta-catenin and HIF1 level of mRNA levels in the serum of patients with endometriosis in comparing to controls. Experimental group (n = 60), consisting of patients with histologically recognized endometriosis. After isolation of the total mRNA from the peripheral blood and following reverse transcription into cDNA, was the level of expression of the genes studied by the method qRT-PCR using the housekeeping gene Gapdh, and Hprt.

In the peripheral blood of patients with assumed but histologically not confirmed endometriosis was detected non-significant increase in the mRNA levels of both genes compared to the control. mRNA levels for HIF1 was about 9% and for the beta-catenin about 21% higher in comparing to the control group. In the group with confirmed endometriosis was found that HIF1 had level about 50% higher than the controls, and beta-catenin mRNA level increased by 56%. Study of detailed understanding of the mechanisms of specific markers transcription is highly current topic, and can lead to the development of new diagnostic and therapeutic applications in monitoring and treatment of patients with endometriosis.

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Abstract number 0202

Proteomic validation of biomarkers for discrimination of benign and malign prostatic hyperplasia

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Background: Serum protein profiles were analyzed in order to discriminate between prostate cancer (PCa) and benign prostatic hyperplasia (BPH).

Methods: Histological specimens were obtained performing trans-rectal ultrasound guided prostate biopsy (TRUS) in the patients in order to identify PCa, BPH and inflammation. Surface Enhanced Laser Desorption/Ionization-Time of Flight-Mass Spectrometry (SELDI-ToF-MS) and two-dimensional gel electrophoresis (2-DE) coupled with Liquid Chromatography-MS/MS (LC-MS/MS) were used to analyze serum samples from patients with PCa and BPH.

Results: SELDI-ToF-MS analysis of serum samples did not show differences in protein profiles between PCa and BPH. Differences became evident when the presence of inflammation was taken into consideration. When samples with histological sign of inflammation were excluded, 20 significantly different protein peaks were detected. Subsequent comparisons (comparison of PCa with and without inflammation, and BPH with and without inflammation) showed that 16 proteins were differently expressed in the presence of inflammation, while 4 protein peaks were not modified. With 2-DE analysis, comparing PCa without inflammation vs PCa with inflammation, and BPH without inflammation vs BPH with inflammation, 29 and 25 differentially expressed protein spots were identified, respectively. Excluding samples with inflammation, the comparison between PCa vs BPH showed 9 unique PCa proteins, 4 of which overlapped with those previously identified in the presence of inflammation, while other 2 were proteins, not identified.

Conclusions: This study indicates that inflammation might be a confounding parameter for proteomic biomarkers of PCa. The results indicate that only a well-selected protein pattern should be considered as a potential biomarker of PCa.

Abstract number 0297

Importance of the detection of three SNPs in DPYD gene to reduce severe toxicity induced by 5 fluorouracil treatment in cancer patients

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Background: Dihydropyrimidine dehydrogenase (DPD) activity is subjected to a wide functional variability due to a large number of SNPs and this variability may be responsible of toxicity in cancer patients during the treatment with the anticancer drug 5-fluorouracil (5-FU). Presence of specific SNPs in this gene is responsible for a broad range, from partial (3-5% of population) to complete loss (0.2% of population) of enzyme activity.

Methods: Herein we report our experience in the use of a specific Mass Spectrometry based approach (Myriapod® ADMET panel, Diatech), for the detection of three DPYD variants: IVS14+1G>A (rs67376798); c.1679T>G (rs55886092) and c.2846A>T (rs67376798) in 404 cancer patients, collected in 8 months, to prevent chemotherapy induced toxicity.

Results: Analysis revealed the presence of DPYD variants in 9 patients. Among them 6 evidenced c.2846A>T, and 3 carried the IVS14+1G>A variants. The SNPs c.1679T>G was not evidenced.

Conclusions: Considering the large number of patients treated each year with 5-FU or other FPs, and the human and economical cost of grade 3 and 4 toxic side effects, genetic characterization at least of the most important polymorphisms should be considered mandatory. Furthermore, to maximize to informative role of genetic testing in DPYD gene, more efforts are requested in order to define the most correct panel of SNPs that should be analyzed to prevent chemotherapy induced toxicity.
Impact of occupational exposure to lead and cadmium on antioxidants in automobile technicians in Ibadan, Nigeria

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Incidence of occupational exposure to lead and cadmium is a persistent feature with consequent health implications amongst automobile technicians especially in developing countries. Generation of free radicals through this exposure has been implicated in the attendant pathologies. Blood Lead Levels (BLL), Urinary Lead (ULL) and Cadmium Levels (UCL) in comparison with oxidative stress status in automobile technicians in Ibadan were evaluated 65 apparently healthy volunteers recruited for the study. Cases were occupationally exposed automobile technicians (panel beaters, automechanics, battery repairers and autopainters) while controls were non-occupationally exposed individuals all resident in Ibadan. Total Antioxidant Capacity (TAC) and Total Plasma Peroxide (TPP) were evaluated while urinary lead and cadmium levels were analyzed using standard laboratory methods.

Increased TPP level and Oxidative Stress Index (OSI) were observed in cases compared to controls ($p < 0.05$) and $p < 0.05$ respectively). The observed reduction in TAC, in automobile technicians was not significant ($p = 0.056$). There were also significant increases in BLL, urinary lead and urinary cadmium levels ($p < 0.05$, $p < 0.05$ and $p < 0.05$ respectively). The BMI, systolic pressure and diastolic pressure were similar between cases and controls. Exposure to lead and cadmium which may be the aetiological basis of the antioxidant/oxidant imbalance resulting in oxidative stress was established in this study.

Protein sulphydryl groups (PSH) as early oxidative stress biomarker in mild chronic obstructive pulmonary disease (COPD) and asthma

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Chronic obstructive pulmonary disease (COPD) and asthma are obstructive airway disorders characterized by heterogeneous chronic airway inflammation and oxidative stress. Many observations suggest that oxidative stress plays an important role in airway diseases, increasing the severity of the pathology and reducing lung function. So the aim of this study was to investigate if some oxidative stress biomarkers changed early during the onset of these pathologies. We compared patients with mild stable COPD ($n = 29$) and mild stable asthma ($n = 24$) and two different control populations, matched on the basis of gender, age and smoking status. Plasmatic biomarkers of oxidative stress, as GSH, PSH, taurine, paraoxonase activity 1, TBARS and ergothioneine, and lung function tests were measured in all subjects. The forced expiratory volume in 1 second (FEV1) is an important spirometric parameter, which is an index of lung obstruction, and it was associated with age both in patients and control groups. Results obtained from this study show that FEV1 was positively correlated with PSH in COPD patients ($\rho = 0.49$, $P = 0.007$) and that lower PSH was the only oxidative stress biomarker independently associated with increased odds of both COPD ($OR = 0.50$, 95% CI 0.26–0.95, $P = 0.03$) and asthma ($OR = 0.32$, 95% CI 0.13–0.78, $P = 0.01$). These results suggest that protein sulphhydrylic groups are a sensitive and early oxidative stress biomarker in mild COPD and asthma.
Possible role of oxidative stress in the pathogenesis of alopecia areata

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Background: Alopecia areata (AA) is a chronic, inflammatory and autoimmune disease, presenting with non-scarring hair loss. Although the precise etiopathogenesis of alopecia areata remains unknown, oxidative stress is thought to play a role.

Objective: The aim of this research was to investigate the role of oxidative stress in AA by measuring the levels of plasma malondialdehyde (MDA) and the activities of erythrocyte catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx).

Material and methods: The study included 24 AA patients and a control group consisting of 24 age- and sex-matched healthy volunteers. The levels of plasma MDA and the activities of erythrocyte CAT, SOD and GPx were measured and compared in both groups.

Results: Plasma MDA levels were significantly increased (P < 0.05) in patients with AA compared with controls. No significant difference between erythrocyte SOD activities was observed between patients and controls (P = 0.108). The mean erythrocyte GPx and CAT activities were significantly reduced (P < 0.05 and P < 0.05 respectively) compared with the control group.

Conclusion: Patients with AA displayed reduced erythrocyte CAT and GPx activities and enhanced plasma MDA levels. These results demonstrate the presence of an imbalance in the oxidant-antioxidant system and support the concept of a possible role of oxidative stress in AA pathogenesis.

Preanalytical phase

Can provide stability information the red blood cell research parameters?

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Introduction: The increasing automation of clinical laboratories and the centralization of sample analysis to reference laboratories to cover large geographic areas represents a change in conditions. As a result, the storage time of the blood before to be analyzed has increased and some changes can be perceived in several parameters of the hemogram. The aim is to assess the stability of the red blood cell (RBC) research parameters together with other conventional parameters on the Sysmex XN-series, under the usual routine conditions of a Core laboratory.

Methods: During 6 months 220 whole blood samples were processed at different hours (0h/2h/4h/8h/16h/12h). The change in the concentration of parameters at each of the times (Yt) compared to the initial value (Y0) was expressed as a percentage change (Yt% = (Yt/Y0)*100) and the mean percentage change (Ymt%) was calculated. Stability was evaluated according to 3 criteria. Metrology criteria according to between-batch (CVb,) and within-run (CVw) analytical variation, by which Ymt% could not exceed the minimum significant change (MSC = ±1.65*CV). Biological criteria based on intra-individual biological variation (CVD), by which Ymt% could not exceed the desirable significant change (DSC = ±0.5*CVD).

RESULTS: RBC and reticulocyte counts were considered stable until 12h. Mean Corpuscular Volume, Hypo-haemoglobinised red cells% (HIPO-RBC), Macrocytic-RBC% and Fragmented-RBC% (FRC) do not accomplish several criteria. Micro-RBC and reticulocyte production index (RPI) are not stable later than 4-6hours.

Conclusions: Results suggest that the most affected parameters were the Macrocytic-RBC, HIPO-He and FRC. RPI after 6h cannot provide information of the bone marrow recovery capacity.

Evaluation of the rapid serum tube for haemolysis index in the emergency department

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Aim: The aim of this study was to compare rapid serum tube (RST) with the widely used serum separator tube (SST) and K2EDTA tube for haemolysis, lipemia and icterus indexes.
Methods: Becton Dickinson (BD; Franklin Lakes, NJ) RST, SST and K2EDTA tubes were collected in tandem from 75 patients admitted to Emergency Department over a 1 month period. Haemolysis, lipemia and icterus indexes were performed on Architect c8000 (Abbott Laboratories, Abbott Park, IL) analyser using a spectrophotometric method measured at multiple wavelengths. Statistical significance of the data was determined by the Wilcoxon signed rank test. P-values <0.05 were considered to be significant.

Results: The rate of haemolysis was %58.7 (44/75) in RST, %50.7 (38/75) in SST and %72 (54/75) in plasma specimens. The results from the RST specimens were comparable with those from the SST specimens on haemolysis, lipemia and icterus indexes. There was statistical significance in haemolysis and lipemia indexes between the SST and plasma specimens and in lipemia indexes between the RST and plasma specimens (p<0.05).

Conclusion: The RST specimens provide acceptable performance compared with the other specimens. In order to reduce the turnaround time on emergency department, using the RST will be convenient.

Abstract number 0029

The difference between serum and plasma intact parathyroid hormone concentrations

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Aim: Intact Parathyroid Hormone (iPTH) is an 84 amino acids length polypeptide hormone that is secreted by parathyroid gland. iPTH concentrations may be variable in different biological samples. In this study, we compared iPTH concentrations of the serum samples we used in routine clinical practice with concentrations of the fresh cold plasma samples.

Methods: Serum and plasma samples of 53 patients who presented to our institute were included in the study. Blood samples were collected using the BD Vacutainer (®) K2EDTA tubes and BD Vacutainer (®) Serum Separator II Advance Tubes (SST II) in sequence. iPTH concentrations were performed on i2000SR analyser (Abbott, IL, USA). The difference between serum and plasma concentrations were evaluated by student t-test and linear regression analysis.

Results: The mean value of plasma concentrations was 83.25 pg/mL (ranged from 5.20 to 260.10) and the mean value of serum concentrations was 78.85 pg/mL (ranged from 5.00 to 247.80). There was a statistically significant difference (p<0.001) and strong correlation (r2=0.979) between serum and plasma concentrations.

Conclusion: The reference intervals that stated on the kit inserts are usually calculated by using plasma samples as on our kit insert. However, both serum and plasma samples can be used to measure iPTH concentrations. Clinical laboratories should know that, especially, it is crucial for the results that are near the upper reference limit, how their measurement method is affected by using serum or plasma samples.

Abstract number 0038

Stability study of leukocyte research parameters on the Sysmex-XN

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Introduction: Inadequate conditions of transport and storage of samples can generate unreliable results that do not reflect the real clinical situation. Aims: Compare the results obtained in the Leukocytes and Nucleated cell count parameters according to the processing hour and the channel of cytometry employed (WDF/WNR/WPC); Asses the neutrophils research parameters at different hours that evidence quantitative variations of the intensity dispersion signal related with the neutrophils structure.

Methods: During six months 300 whole blood samples were processed from 0h-12hours (interval of 2hours). The change in the concentration of parameters at each of the times (Yt) compared to the initial value (Y0) was expressed as a percentage change (Yt%=Yt/Y0*100) and the mean percentage change (Ymt%) was calculated. Stability was evaluated according to 3 criteria: Metrology criteria according to between-batch(CVb,) and within-run(CVw) analytical variation, by which Ymt% could not exceed the minimum significant change (MSC=±1.65*CV). Biological criteria based on intra-individual biological variation(CVD), by which Ymt% could not exceed the desirable significant change (DSC=±0.5*CV).

Results: Leukocytes and Nucleated cell count performed by WNR and WDF channels are considered stable according to MSC and DSC. Nevertheless WPC channel does not accomplish any stability criteria at several time of analysis. Neutrophil count is stable, but the intensity of the Neutrophil-SSC(granularity) and Neutrophil-SFL(size) signals are not stable later than 6hours according CSMw.

Conclusion: Results suggest better conditions of stability in WNR and WDF channels and could explain with quantitative variations that some of the neutrophils morphological changes are time dependent.
Abstract number 0046

Suitability of a novel urine collection tube for microbial testing

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Background: Reliable urine testing results are of utmost importance for diagnosis, monitoring and therapy of patients with urinary tract diseases. An increase in microbial counts due to a missing preservative or high transport temperatures may lead to false results due to transportation delays. The VACUETTEŻ Urine CCM Tube contains a novel preservative stabilizing urine samples for microbiology at room temperatures for up to 48h. Materials: A study evaluated urine samples (total n=170, partly spiked) from clinically inconspicuous and conspicuous (nitrite and leukocyte positive with dipstick urinalysis) urine specimens. Those samples were collected in the new urine tube. The microbiological cultures were generated on the same day within 2h after collection, after 24h and 48h. All specimens were stored at 20–25°C between the time points. Stability testing was done with the following organisms: Escherichia coli, Enterococcus faecalis, Pseudomonas aeruginosa, Staphylococcus saprophyticus, Proteus mirabilis, Candida albicans. Results: According to the performance criteria, the starting values do not differ significantly from the reference tube and the results after storage for 48h at room temperature do not differ significantly (one log step) from the 0-2h results, the stability of the pathogens could be demonstrated without significant differences in comparison to the reference tube. Conclusion: The CCM Tubes are suitable for stabilizing the tested organisms responsible for urinary tract infections for 48h at room temperature. It is a urine sampling and transport system suitable for microbiologic diagnostics and is found to be useful in improving preanalytics in urine culture testing.

Abstract number 0057

Glucose and albumin quantification are potential indicators of the stability of long-term stored samples for glycated albumin measurement?

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Monitoring the glycemic status is most important for cardiovascular risk estimation. Glycated albumin (GA%) is receiving growing interest as a biomarker which could support clinician’s efforts for diabetes management and to prevent long-term cardiovascular-related complications. For research purposes, samples stored at -20°C for more than 6 months seem not suitable for GA% quantification due to a time-dependent increase in GA% level.

Our aim is to assess whether stored samples are suitable for GA% quantification, exploring the useful of glucose (Glu) and albumin (Alb) as indicators of stability. We quantified GA%, Alb and Glu levels on 60 serum samples of healthy non-diabetic individuals (20 males and 40 females; mean age: 38.3±12.47 years; body mass index: 25.94±2.46 kg/m²) stored at -20°C for 3.64±0.57 years (T1). Alb and Glu values at drawing time (T0) were also available. GA% was quantified using QuantILab Glycated Albumin assay.

Mean GA% was 31.69±5.77. Compared to T0, both Alb and Glu decreased at T1 (-25% and -16%, respectively; p<0.001 for both). GA% correlated inversely with Alb at T1 (-0.286, p=0.027) and directly with storage time (0.357, p=0.005). No correlations were observed with Glu and overt time changes in Glu and Alb (T0-T1).

Obtained GA% values are not reliable, being typical of diabetic individuals. Alb instability in samples stored at -20°C seems to be one of the main factor affecting GA% quantification and could enforce previously published data.

A suspicious decreased value of Alb compared to time of freezing may suggest that stored samples are inadequate for GA% quantification.

Abstract number 0105

Study of interchangeability in hemolysis index

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Introduction: Hemolysis may produce interference in many laboratory tests. Laboratories detect and reliably quantify hemolysis in every collected sample by automated estimation of the Hemolysis Index (HI). Despite this advantage, a degree of variability still affects the HI estimate,
and therefore the harmonization of HI results from different analytical systems should be a priority. Different analyzers may report different HI estimation and this may not only change the corrective actions taken, but also, and more importantly it may influence patient care.

The aim is to compare the HI estimated by our analyzers to ensure patient safety.

Methods: A pool of samples was used with HI 2614 mg Hb/dL, serial dilutions were performed and HI was determined. The dilutions were processed in four Architect ci6000 (A1, A2, A3, A4) and a ci6200 (A5) autoanalyzers. The method to determine the HI was bichromatic spectrophotometric readings and calculated using specific algorithms (Abbott).

Results:

<table>
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<tr>
<th>Dilution</th>
<th>A1</th>
<th>A2</th>
<th>A3</th>
<th>A4</th>
<th>A5</th>
<th>Mean</th>
<th>% Maximum difference</th>
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<td>683</td>
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</tr>
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Conclusions: We define a difference <10% as acceptable. The difference between instruments was lower. The laboratories need guidelines prepared by leading scientific organization recommending the best approaches. The implementation of an external control could help improve the decision-making.

Abstract number 0258

The relevance of sampling time in TSH determination

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Background: TSH is optimal test for diagnosing, assessing all phases of thyroid dysfunction and reflecting thyroid status when euthyroid state has been reached. The TSH displays circadian biorhythm and shows decrease during the sampling period in our laboratory (7:00-9:00), since we have 250-300 patients every morning notwithstanding well organized system of collecting blood samples from general practice physicians. The aim of this study was to determine percentage of decrease in TSH values during sampling period that might effect results and, consequently, diagnostic decision.

Materials and Methods: The study enrolled 2 groups of healthy subjects (33 subjects (group1) and 14 subjects (group2)). Samples were drawn at 7:00, 8:00 and 9:00 for group 1 (sampling period), and for group 2 (TSH daily profile) at 7:00, 11:00, 17:00 and 21:00. TSH was determined by electrochemiluminiscence method performed on cobas-e601 (Roche-Diagnostics). Expected values (2.5-97.5th percentiles) were 0.270-4.200 mIU/L.

Results: Mean percentage of decrease in individually TSH values 7/9h, 7/8h and 8/9h (group1) were 31.51% (5.5-51.8%), 21.96% (3.7-45.6%) and 14.01% (1.9-29.3%), respectively. Mean values at 7:00, 11:00, 17:00 and 21:00 (group2) were 3.126 (1.030-6.000), 2.028 (0.871-4.050) and 2.953 (1.080-5.630), respectively.

Conclusions: Regarding the significant decrease in TSH levels (up to 51.8%) during sampling period, the blood collection should be carried out around same time, if possible, especially for therapy monitoring. That could be achieved by blood sampling at general practice physicians or sampling between 8:00-9:00. According to the data of TSH daily profile sampling time has to be obeyed.

Abstract number 0313

Cost analysis of preanalytical errors in terms of samples drawn for blood gas analysis

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Government of Health’s political sanctions has integrated the lab tests into outpatient clinics’ patient packages which led the hospital managers to see the laboratory tests as a cost increase. The aim of our study was to analyze the cost of preanalytical errors due to wrong blood draws which is leading to sample rejection. We analyzed last year’s rejection rates for blood gas analysis using our laboratory information system.
The costs were calculated by use of last tender’s prices given to our hospital. Number of blood gas analysis done in the last year was 46619 and the number of rejected samples was 5119 (10.5%). Analysis of re-requesting after rejection has revealed us that 45-50% of the rejected samples were not requested again. This shows that half of 5119 samples (2500 samples) were not requested after rejection. This has lead to an increase in costs of blood gas analysis around 8000 TL (2600$) in total. When divided to the total cost every blood gas analysis test has increased in price around 0.18 TL (0.06$). When all of the rejected samples are taken into consideration it is apparent that too much money is spent every year due to preanalytical errors.

Abstract number 0315

Comparison of BD SST™ and RST™ tubes with Sarstedt S-Monovette® serumgel tubes in terms of hemolysis resulting from blood draw

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Hemolysis is one of the leading pre-analytical problems encountered especially in emergency rooms. This study is done to see the effect of different tube types on the hemolysis ratios encountered in the yellow treatment room of our hospital. Blood were drawn in Becton Dickinson SST™ and RST™ tubes and Sarstedt S-Monovette® Serum Gel tubes concurrently from 128 patients admitted to the yellow area of our emergency room. We used the aspiration method by use of Multiadaptor for S-Monovette® connected to the established vascular access to fill the S-Monovette® Gel Tube. On the same patient we used the vacuum method by use of Vacutainer® Luer-Lok™ connected to the established vascular access to fill Vacutainer® SST™II ve Vacutainer® RST™ tubes. Hemolysis was determined via the serum index analysis on Roche Cobas 6000 analyzers. In 3 patients hemolysis was encountered in both tube types and were regarded as in vivo hemolysis and rejected from the study. As a result the percentage of hemolysis encountered in Sarstedt vs BD-SST and Sarstedt vs BD-RST were 4.41% vs 14.71% and 0% vs 18.97%, respectively. The number of tubes having hemolysis over the acceptable limit of hemolysis which is 50 mg/dL in Sarstedt vs BD-SST and Sarstedt vs BD-RST were 5 vs 12 and 1 vs 12. In our study we saw that vacuum method is more prone to hemolysis effect in emergency rooms. In conclusion more studies should be done to see the effect of vacuuming and aspirating methods on hemolysis problems in emergency rooms.

Biomarkers

Abstract number 0124

A biomarker panel in acute skin graft-versus-host disease

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Acute graft-versus-host disease (aGVHD) is a leading cause of morbidity and mortality following allogeneic haemopoietic stem cell transplant. The study’s aim was to evaluate the clinical utility of a biomarker panel to diagnose and/or differentiate severity at the time of aGVHD onset and identify individuals at risk of developing aGVHD at day0 and day7 post-transplant.

This retrospective study included 11 skin biopsy confirmed aGVHD patients and 17 matched (for; age, gender, disease status, times after transplant and/or time of onset) without aGVHD. Samples were stored at -80C until analysis for Elafin, Regenerating islet-derived 3-α (REG3α), soluble TNF receptor 1 (sTNFR1), Soluble interleukin-2 receptor-α (sIL-2Rα) and Hepatocyte growth factor (HGF) by ELISA.

The composite panel differentiated between non-GVHD and aGVHD at day0 and day7 post-transplant using formulae: [1000x{constant - (86.55xElafin) + (1199x sIL-2Rα) - (91796x sTNFR1)}] and [1000x{constant - (37.75xElafin) + (397.93xHGF) - (29.67xREG3α)}] respectively. However, they did not differentiate between non-GVHD and GVHD groups.

The panel [1000x{(-39444) + (47.23xElafin) + (314.96x sIL-2Rα) + (128.79xREG3α)}] differentiated between non-GVHD and GVHD and the panel [1000x{constant - (33.63xElafin) - (247.78x sIL-2Rα) - (212.29xREG3α)}] categorised grading of aGVHD at time of onset. The area under curve for the panel was 0.65 with specificity, sensitivity, positive predictive value and negative predictive value of 100%, 55.6%, 100% and 78.9% respectively (p = 0.03).

Our pilot data supports the use of a biomarker panel in the work-up of acute skin-GVHD. Further larger studies are needed to validate its use in the clinical management of aGVHD.
Abstract number 0156

Bone turnover markers and TSH in women with osteoporotic fractures

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Background: Osteoporosis and associated fractures are particular risk for postmenopausal women. The aim was to investigate the effect of TSH on bone turnover in women with osteoporotic fractures.

Methods: The study group consisted of 44 postmenopausal women, admitted to the Department of Orthopaedics and Traumatology at the University Hospital due to osteoporotic fracture. Study group was divided in subgroups: postmenopausal and senile osteoporosis. Reference group consisted of 32 women without fracture. All women were characterized by a normal thyroid function. In both groups concentration of TSH, free thyroxine (fT4), 25(OH)D, carboxy-terminal telopeptide of type 1 collagen (CTX) and amino-terminal propeptide of type 1 procollagen (PINP) were measured in serum.

Results: Patients with fractures had lower TSH ($p \leq 0,02$) and vitamin D ($p \leq 0,0001$) concentrations as compared with women without fracture. Women from study group were characterized by higher CTX concentration ($p \leq 0,02$). In study group a negative correlation between age and vitamin D was observed ($r = -0,46$). In both, study and reference group a significant correlation between bone resorption and bone formation markers was found ($r = 0,57$; $r = 0,75$, respectively). No relationship was observed between TSH concentration and bone formation and resorption markers in all groups. Women with postmenopausal and senile osteoporosis had lower vitamin D ($p \leq 0,0003$), higher CTX ($p \leq 0,03$) concentrations and predominantly TSH concentration in low normal range.

Conclusion: Patients with osteoporotic fractures showed increased bone resorption however, no significant relationship between serum TSH concentration and bone turnover.

Cancer diseases and tumour markers

Abstract number 0097

Reference intervals for the kryptor second-generation chromogranin A (CGA) assay

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Background: CGA measurement is recommended for diagnosis and management of neuroendocrine tumors. The fully automated CGA immunoassay marketed by Brahms to be used on the ThermoFisher Kryptor platform has recently been upgraded with a second-generation method (CGAII) to allow prolonged storage of serum samples at 2-8°C. Here we established reference intervals for CGAII.

Methods: Serum samples were obtained from 122 healthy blood donors [51.6% males; median age (25th-75th percentile), 42.5 years (32.3-51.0)]. Shapiro-Wilk (SW) test was used to assess normality of distribution of CGA values and Wilcoxon rank sum test was used to compare groups.

Results: Although deviating from normal distribution (SW-statistic, $P < 0.001$), the visual inspection of data did not suggest log-transformation. Therefore, we opted for using original data to perform statistical analyses. Median (25th-75th percentile) CGA concentration was 44.3 µg/L (33.6-58.5), with no sex-related difference ($P = 0.27$). Regression analysis including age and sex as covariates confirmed the lack of gender influence ($P = 0.15$), showing, however, that CGA concentrations increase with age ($P = 0.009$). However, according to the very low value of adjusted $R^2$ (0.06) describing the dependence of CGA values from age, we decided to adopt for CGAII clinical use a single upper reference limit (URL) for overall adult population set at the 97.5th percentile of CGA value distribution, i.e., 87.9 µg/L (90% confidence interval: 85.0-90.8).

Conclusions: Our results showed that the obtained URL for CGAII is quite similar to that estimated in previous studies using the first generation assay, implicitly confirming the manufacturer’s statement related to the between-assay result equivalence.

Abstract number 0103

The activity of class I, II, III and IV alcohol dehydrogenase isoenzymes and aldehyde dehydrogenase in bladder cancer cells

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The literature review shows that changes in the alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) activities can play a role in initiation and progression of malignant diseases. The aim of this study was to determine the differences in the activity of ADH isoenzymes and
ALDH in normal and cancerous bladder cells, and in the different grades of disease. The study material consisted of 37 cancerous (15 low-grade and 22 high-grade bladder cancer (BCa)) and 32 histologically unchanged bladder tissues. Class III, IV of ADH and total ADH activity were measured by the photometric method and class I, II ADH and ALDH activity by the fluorometric method with class-specific fluorogenic substrates. The total activity of ADH was significantly higher in BCa (0.22+/−0.32 nmol/min/mg protein) than in healthy subjects (0.08+/−0.06 nmol/min/mg protein). The activity of ADH III isoenzyme was also significantly increased in BCa (2.21+/−1.46 nmol/min/mg protein) as compared to the control group (1.29+/−0.89 nmol/min/mg protein). Significantly elevated total activity of ADH was found in both, low-grade (0.17+/−0.11 nmol/min/mg protein) and high-grade (0.90+/−0.53 nmol/min/mg protein) BCa, and activity of ADH III was significantly higher only in high-grade BCa group (2.33+/−1.53 nmol/min/mg protein) compared to controls. The other classes of ADH tested and ALDH, did not show significant differences in the activities between cancerous cells and healthy bladder. The increased activity of total ADH, especially class III isoenzyme, and normal activity of ALDH in bladder cancer, may be the cause of metabolic disorders in cancer cells, which may intensify carcinogenesis in urinary bladder.

Abstract number 0125

The relationship between BMI and selected biochemical inflammatory factors in breast cancer patients

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Aim: Obesity has been associated with an increased overall risk of breast cancer, especially in postmenopausal women. However, there is still controversy on impact of this relationship on premenopausal breast cancer. The aim of presented study was the analysis of several biochemical inflammatory factors levels in respect to body mass index (BMI).

Material and methods: The determinations of CA 15-3, CEA, CRP, IL-6, suPAR and PAI-1 were performed in the group of 217 breast cancer patients and in the reference group of 47 healthy women.

Results: Breast cancer patients at age greater than 55 years had significantly higher concentrations of CRP, IL-6, suPAR, and also BMI values than younger patients. The concentrations of CA 15-3, CEA, CRP, IL-6, suPAR, and PAI-1 was analyzed in four groups of patients selected in respect to BMI range (≤24.9, 25 – 29.9, 30.0 – 34.9, ≥35). Between these groups there were no significant differences in the concentrations of CA 15-3 and CEA. However, CRP, IL-6, suPAR, and PAI-1 showed significant tendency to the increase with increasing BMI ranges (r = 0.314 p = 0.00001; r = 0.331 p = 0.00001; 0.156 p = 0.0214; r = 0.345 p = 0.000001 respectively). There were no significant differences in percentage of patients in regard to the stage of disease, tumor size, lymph node status, histologic grade, and also estrogens, and HER2 expression between particular BMI ranges.

Conclusion: In breast cancer patients overweight, obesity seems to be an important factor in generating the inflammatory response.

Abstract number 0144

The role of chemokine CXCL8 in esophageal cancer

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Objectives. The novel biomarkers of esophageal cancer (EC) are critically needed to improve the diagnosis and prognosis of EC patients. The aim of our study was to assessed the serum concentrations of chemokine CXCL8, known as interleukin-8 (IL-8) in EC patients and compare these levels with healthy controls as well as classical tumor markers for EC – carcinoembryonic antigen (CEA) and squamous cell cancer antigen (SCC-Ag) and with marker of inflammatory states – C-reactive protein (CRP).

Methods. The study included 20 patients with EC and 20 healthy controls. Serum concentrations of proteins tested were measured using immunoenzyme assays.

Results. The concentrations of CXCL8 were significantly higher in the sera of EC patients in comparison to healthy controls (p=0.023), likewise the levels of CRP (p=0.001). Similar results were obtained for classical tumor markers (CEA and SCC-Ag) concentrations, however these differences were not statistically significant. Moreover, a significant positive correlation was observed between CXCL8 levels and TNM stage of EC (p=0.012) as well as CEA levels (p=0.003), whereas CRP concentrations correlated with depth of tumor invasion (T factor) (p=0.004) and SCC-Ag levels (p<0.001). The activity of ADH III isoenzyme was also significantly increased in BCa (2.21+/−1.46 nmol/min/mg protein) as compared to the control group (1.29+/−0.89 nmol/min/mg protein). Significantly elevated total activity of ADH was found in both, low-grade (0.17+/−0.11 nmol/min/mg protein) and high-grade (0.90+/−0.53 nmol/min/mg protein) BCa, and activity of ADH III was significantly higher only in high-grade BCa group (2.33+/−1.53 nmol/min/mg protein) compared to controls. The other classes of ADH tested and ALDH, did not show significant differences in the activities between cancerous cells and healthy bladder. The increased activity of total ADH, especially class III isoenzyme, and normal activity of ALDH in bladder cancer, may be the cause of metabolic disorders in cancer cells, which may intensify carcinogenesis in urinary bladder.

Conclusion. Our findings suggest the potential role of CXCL8 in the development of esophageal cancer.

Acknowledgement: The study was conducted with the use of equipment purchased by Medical University of Białystok as part of the RPOWP 2007-2013 funding, Priority I, Axis 1.1, contract No. UDA-RPPD.01.01.00-20-001/15-00 dated 26.06.2015.
Abstract number 0158

PIVKA-II levels in hepatocellular carcinoma and control groups

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Study aims. PIVKA-II or abnormal des-carboxylated prothrombin (DCP) is proposed as a more specific marker of hepatocellular carcinoma (HCC) than AFP. We assessed PIVKA-II levels in HCC patients and in two control groups.

Methods. Serum samples from HCC patients (63), from cirrhotic patients without HCC (CP; 76) and from individuals without liver disease (WLD; 114) were assayed for PIVKA-II by an automated chemiluminescent immunoassay (Abbott ARCHITECT) and levels were compared among groups.

Results. HCC patients and CP had a similar male/female ratio (3.50 vs. 2.80) and age (HCC: mean 65.3±8.6 years; median 66; CP: mean 61.8±11.1; median 59), whereas GH were significantly younger (mean 24.2±6.4; median 22) and mainly females (65.8%). PIVKA-II levels are summarized in table; no gender differences were observed in any group. By receiver-operating characteristics (ROC) curve analysis the AUC was 0.77 (95% confidence limits: 0.69-0.85); at 50.9 mAU/mL PIVKA-II had 58.7% sensitivity and 92.3% specificity.

<table>
<thead>
<tr>
<th>Patient group</th>
<th>HCC</th>
<th>Cirrhosis</th>
<th>Healthy controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>N. (Males/Females)</td>
<td>63 (49/14)</td>
<td>76 (56/20)</td>
<td>114 (39/75)</td>
</tr>
<tr>
<td>PIVKA-II mean mAU/mL</td>
<td>1624.80</td>
<td>34.16</td>
<td>9.51</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>5925.66</td>
<td>18.84</td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>73.81</td>
<td>29.57</td>
<td>32.83</td>
</tr>
<tr>
<td>Minimum</td>
<td>9.72</td>
<td>14.81</td>
<td>18.63</td>
</tr>
<tr>
<td>Maximum</td>
<td>30001.00</td>
<td>144.59</td>
<td>58.76</td>
</tr>
<tr>
<td>99th percentile</td>
<td>30001</td>
<td>110.18</td>
<td>57.57</td>
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<tr>
<td>2.5th percentile</td>
<td>17.54</td>
<td>18.93</td>
<td>19.18</td>
</tr>
<tr>
<td>97.5th percentile</td>
<td>25957.57</td>
<td>87.97</td>
<td>55.49</td>
</tr>
<tr>
<td>N &gt; 50.9 mAU/mL</td>
<td>37</td>
<td>6</td>
<td>8</td>
</tr>
<tr>
<td>% &gt; 50.9 mAU/mL</td>
<td>58.7%</td>
<td>7.9%</td>
<td>7.0%</td>
</tr>
</tbody>
</table>

Conclusions. These preliminary data confirm the specificity of PIVKA-II levels for HCC, while levels in cirrhotic patients were not different from those observed in apparently healthy individuals. No significant differences related to age and gender were observed.

Abstract number 0172

Association between methylenetetrahydrofolate reductase gene C677T polymorphism and digestive cancer susceptibility

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Introduction: The 5,10 methylenetetrahydrofolate reductase (MTHFR) is a key enzyme in folate metabolism. The MTHFR C677T polymorphism creates a thermolabile enzyme which decreases enzyme activity, appears to interfere with the phenomena of carcinogenesis by reducing the DNA methylation levels and by monitoring the synthesis of DNA. Numerous studies have highlighted the important role of MTHFR in carcinogenesis.

The aim of this study:
1. Determine the allelic and genotype frequencies of MTHFR C677T polymorphism in patients with gastric and colorectal cancers and healthy controls.
2. Performe estimation of relative risk associated with this polymorphism in digestive cancers, compared to healthy controls.

Patients and methods: 30 patients with gastric cancer (GC), 52 patients with colorectal cancer (CRC) and 101 healthy controls, were genotyped for the MTHFR C677T polymorphism by using the PCR / RFLP method.
Results: Allelic frequencies of MTHFR 677T and MTHFR 677C were 34.92% and 65.07% respectively in the control group, 43.75% and 56.25%, respectively, for patients with CCR and 25% and 75% respectively in patients with a GC.

The odds ratio 677 T/T vs 677 C/C and 677 C/T vs 677C/C were 9.82 (0.91-19.54) (p < 0.05) and 1.4 (0.9-2.3) (p < 0.05) respectively in CRC and 1,23 (p = 0,55) et 0, 62 (0, 23-1, 65) (p = 0, 20), respectively in GC.

Conclusion: Our data have indicated that the C677T MTHFR polymorphism doesn't significantly contribute to the inherited genetic susceptibility to GC, while we have shown some evidence for possible genetic contribution of this polymorphism to the development of CRC.

Abstract number 0180

ALC, absolute number Tregs and percentage of apoptotic cells in prognostication of newly diagnosed DLBCL patients.

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Introduction: Diffuse large B-cell lymphoma (DLBCL) is the most common non-Hodgkin lymphoma. Lymphocyte Treg play an important role in the development of DLBCL and in clinical course of the disease. Recent studies demonstrated that decreased absolute lymphocyte count (ALC) in DLBCL has been associated with a worse outcome.

Objectives: Whether quantitative assessment CD4+CD25+++Foxp3high Treg, ALC and percentage of apoptotic cells can improve prognostication in DLBCL patients.

Patients and methods: We examined 23 healthy people and 25 patients (18 men and 7 women, aged 35-75) with newly diagnosed DLBCL at different clinical stages, before (BT) and after completed treatment (AT). The absolute count of lymphocytes Treg-cells and the percentage of apoptotic cells were assessed by means of flow cytometry.

Results: We demonstrated that significantly lower level of ALC and the percentage of apoptotic cells have been observed exclusively in DLBCL patients with high-risk disease (HR) (ALC: BT 1,28±0,47x10³/µl, AT 2,05±0,39x10³/µl, control 2,89±0,89x10³/µl; apoptosis: BT 2,43±0,78% control 1,02±0,45% control 8,42±1,72%). We also showed, that in comparison with patients into low-risk (LR) groups, there is a significant decrease in the absolute number of Tregs (BT: 0,07±0,008x10³/µl, 0,02±0,001x10³/µl, 0,04±0,001x10³/µl, AT: 0,09±0,004x10³/µl, 0,03±0,002x10³/µl, 0,07±0,004x10³/µl; control 0,1±0,02x10³/µl).

Conclusions: These results suggest that lymphopenia, the decreased absolute number of Tregs, and a percentage of apoptotic cells, correlates with clinical staging in DLBCL patients. The decreased level of Tregs and apoptotic cells after treatment might predict a poor clinical outcome in patients treated with R-CHOP (cyclophosphamide, doxorubicin, vincristine, prednisone, rituximab, premedication).

Abstract number 0181

The plasma levels and diagnostic utility of MMP-7, CA 125 and HE4 in ovarian cancer patients

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4 Esthetic Medicine, Medical University of Białystok, Poland

Background: Epithelial ovarian cancer (EOC) is one the most important cause of cancer death with physical signs in advanced stages and poor 5-year survival. Identification a highly sensitive and specific biomarkers panel with potential diagnostics utility in this malignancy is urgently needed promising alternatives to ultrasound examination.

Patients and methods: The study included 120 EOC patients (serous and endometrioid sub-types). The control groups comprised 80 benign ovarian tumor patients (cystis serous or cystis endometrioides) and 70 healthy volunteers. Plasma MMP-7 levels were determined by ELISA, HE4 and CA125 levels by CMIA method.

Results: Plasma levels of MMP-7 (7,92 ng/ml), CA 125 (707,9 U/ml) and HE4 (636,85 pmol/L) were significantly higher in EOC patient in comparison to the healthy (p<0,001 in all cases) and ovarian cysts groups (p<0,001 in all cases). All the biomarkers specificities received high values (equal to 94%). The MMP-7 diagnostic sensitivity (62%), positive and negative predictive values (93% and 54%) were similar to those of CA 125 (65%; 94%; 58%) or HE4 (66%; 94%; 52%).

Conclusions: Obtained results suggest a high diagnostic potential of MMP-7 in combined panel with conventional tumor markers in EOC patients diagnostics.
Abstract number 0292

Patients suffering from metastatic prostate cáncer, increase in survival, accompanied with quality of life

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34 patients over 65 years old, all of them suffering from prostate cancer, with radical prostatectomia without surgical castration and undergoing treatment with zoladex (goserelin) were studied.

At the start of this study all the patients presented moderate to severe anemia, moderate leucocitosis, severe thrombocytopenia, accompanied with macrocitic elements, very high levels of plasmatic homocystein, hepatic profile, CPK, LDH, prostatic specific antigen above normal.

The patient’s were treated with ferrous fumarat and folic acid and with a dose of acenocoumarol, also an appropriate dose of prednison to avoid hepatic disorders.

At hematological control of the following semester it was clear-sighted that slowly and gradually all the hematological values, including the hepatic profile and prostatic specific antigen were encouragingly changing, that’s why it was decided to continue with acenocoumarol, a mimimun dose of prednison and an intradermic injection of zoladex every exact 40 days instead of every 21 days as it is suggested in the therapeutic antitumoral, and the new diet to do the corresponding supply.

At the end of four years, the patients reached the analytical values expected, the plasmatic homocystein had decreased to normal level, accompanied with normocitic elements, together with the hepatic profile stabilization, CPK and LDH and the decline of indetectable of prostatic specific antigen.

The patients in this study, subjected to the treatment given, have been able to improve their life-forecast, considering that their survival time has increased, which added to their rights to enjoy a better quality of life as longeuous patients allows gaining psycho-social stability.

Abstract number 0335

Utility of laboratory parameters in the differential diagnosis of limited vs extensive small cell lung cancer disease

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Introduction: In cancer patients it is often assumed that there exists a dynamic system consisting of cancer and the host organism. Studies of tumor markers provide information about tumor stage, whereas calculated on the basis of laboratory parameters of inflammation indices characterize failure of host homeostasis. The aim of this study was to analyze the usefulness of selected tumor markers and calculated indices in the differential diagnosis of early or advanced stages of SCLC.

Material and methods: Hematological parameters and CRP, albumin, prealbumin, alpha-1 acid glycoprotein as well as tumor markers were measured in 100 LD-SCLC and 50 ED-SCLC patients.

Results: In comparison to LD-SCLC, in ED-SCLC patients were observed significantly higher concentrations of NSE and ProGRP and also higher values of NLR, PINI, CSI and index PS. Significantly lower values of INI, PINI and NRI at the lack of differences for PLR and BMI were found. The evaluation of the usefulness of the studied indicators in the differential diagnosis of ED vs. LD based on the analysis of AUC ROC curves. The larger AUC’s for ProGRP (0.687) and NSE (0.664), as well as for INI (0.693), PINI (0.695), index PS (0.685), CSI (0.664) and NLR (0.642), confirmed the relatively high utility of tumor markers and calculated indices in the differential diagnosis. For remaining indices were showed lack of usefulness for the assessment of disease advancement.

Conclusion: The tumor markers and calculated indices results may bring useful information, from the clinical point of view, for the differentiation of disease advancement.

Abstract number 0336

The importance of HE4 serum concentration for the treatment management decisions in patients with invasive bladder cancer after transurethral electroresection

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Background and purpose: The treatment of patients with invasive bladder cancer is a multistage process, requiring consideration of a number of clinical factors, including radicality of transurethral resection of the bladder tumor (TURBT). The aim of the study was to evaluate the usefulness of HE4 concentration in qualification for conservative or palliative treatment in patients with invasive bladder cancer after TURBT.

Methods: The serum HE4 measurement was performed in 136 patients with invasive bladder cancer after TURB (radical or non-radical) qualified for conservative or palliative treatment, and in the reference group of 30 healthy controls.

Results: In patients with bladder cancer HE4 concentration was significantly higher than in the reference group. There was a significant relationship between HE4 serum concentration and bladder cancer stage, performance status, hydronephrosis. The histological grading did not show such significant correlation.

Conservative treatment was performed in 98.2% of the patients with radical TURBT and in 51.4% of patients with non-radical TURBT. HE4 concentration was significantly higher in non-radical TURBT patients not qualified for conservative therapy than in patients receiving this type of treatment (p = 0.00001). Based on the analysis of the ROC curve designated for non-radical TURB patients disqualified from conservative treatment, compared to patients undergoing this form of therapy, the optimum cut-off concentration of HE4 was determined as 171.5 pmol/L (sensitivity - 67.9%, specificity - 83.1%).

Conclusion: In patients with invasive bladder cancer, determination of HE4 concentration can be helpful in qualifying non-radical TURB patients for radical conservative treatment.

Abstract number 0341
The clinical utility of serum Se, Zn, Cu and plasma TAS determination in colorectal cancer and adenoma in Polish population

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Selenium (Se), zinc (Zn) and copper (Cu) are considered to be involved in carcinogenesis, also of the colon and indirectly participate in the defence against reactive oxygen species (ROS). Oxidative stress is thought to play a pivotal role in tumorigenesis, also of the colonic mucosa. Plasma total antioxidant status (TAS) evaluates the plasma ability to remove ROS and to prevent their further generation.

The aim of the study was to compare clinical utility of serum Se, Zn, Cu and plasma TAS determination in colorectal cancer and adenoma in the population of low Se and borderline Zn status.

On the base of clinical examination and colonoscopy/histopathology, the patients (n=79) were divided into: colorectal cancer (CRC, n=30) or adenoma (CRA, n=19) groups and healthy controls (CTRL, n=30).

Serum Se concentration was lower in CRC (0.84 µmol/L) than in both CRA (0.92 µmol/L) and CTRL (0.91 µmol/L). Serum Zn concentration was decreased in CRA (13.01 µmol/L) when compared to CTRL (13.70 µmol/L). Plasma TAS was significantly lower in CRC group (1.15 mmol/L) than in CTRL (1.47 mmol/L). Se concentration positively correlated with TAS in all studied patients. ROC curve analysis revealed that Se level was of the highest diagnostic utility for the discrimination of CRC from both CRA and CTRL. Zn and TAS levels were also of significant accuracy in the differentiation between studied groups of persons.

We propose that individualised cancer risk approach should comprise plasma/serum Se, Zn and TAS assays.

Case studies

Abstract number 0068
Mirizzi syndrome type-II 3

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Introduction: Mirizzi syndrome is a rare cause of obstructive jaundice caused by extrinsic compression of the common hepatic duct in gallstone patients. The elevation of carbohydrate antigen 19.9 (CA19.9) has a 90% sensitivity for pancreatic and 70% for biliary cancer.

Objective: We present the relationship between the Mirizzi syndrome type-II and persistent elevation of CA19.9.

Methods: A 82-year-old woman was admitted due to weight loss (10kg/year), asthenia and anorexia. Her clinical records included former smoking and alcohol abuse. Physical examination showed significant cognitive impairment. She had elevated aminotransferases, amylase, total bilirubin and CA19.9 (224 U/mL; normal: <37 U/mL).

The chest X-ray was normal. Abdominal ultrasound and retrograde endoscopic cholangiopancreatography showed great dilatation of the intrahepatic bile duct due to large choledocholithiasis, previous papilotomy and placement of a biliary drainage. In the abdominopelvic-CT, correct placement of stent bile was checked. She was cited for elective cholecystectomy.

Results: Cholecystectomy was performed, observing a large choledocholithiasis shocked (2cm) and cholecysto-hepatic fistula, findings suggestive of Mirizzi syndrome type-II. Gallstones were removed by cholecysto-jejunostomy Roux-Y. Biochemical values normalized in the immediate postoperative period, except for CA19.9, which took 6 months to lower, being 59 U/mL in the last analytical control. The patient remains asymptomatic.

Conclusions: In the differential diagnosis of obstructive jaundice, elevations of CA19.9 values should be interpreted with caution. Benign processes like the Mirizzi syndrome type-II can simulate malignant biliary processes with serum elevations of CA19.9, with persistence over time despite the resolution of biliary obstruction as in the case presented.

Abstract number 0132

Primary plasma cell leukemia. Clinical case report.

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Hospital Universitario Donostia, Spain

Background-aim: Plasma cell leukemia (PCL) is a rare and aggressive variant of multiple myeloma (MM), characterized by the presence of plasma cells (PC) in peripheral blood (>20% or >2,000/µl).

Methods: 51-year-old woman presented to the emergency department with acute abdominal pain and neck pain, started four months ago.

Results: Creatinine 0.94 mg/dl (0.4-1.0), sodium 133 mEq/l (135-145), chloride 90 mEq/l (93-110), calcium 10.6 mg/dl (8.6-10.2), total protein 10.5 g/dl (6.6-8.7). Hemoglobin 8.5 g/dl (12-15.3), leukocytes 12.46*10³/µl (3.8-10), monocytes 31% (3.5-12) and 3.88*10³/µl (0.2-0.9). Flags with blood-smear review: monocytosis, blasts and atypical lympho. We observed 21% of PC.

Supplementary tests:
- Immunoglobulins: IgA 49.73 g/l (0.7-4), IgG 1.17 g/l (7-16), IgM 0.11 g/l (0.4-2.3). Free light chains: κ 2.64 mg/l (3.0-19.40), λ 1.897.18 mg/l (5.71-26.30), \( \kappa/\lambda \approx 0.8 \). - β2-microglobulin: 11.97 mg/dl (0.8-2.2)
- Proteinogram and immunofixation: IgA-λ (5.59 g/dl) in beta fraction. In gamma, λ light chains (0.1 g/dl). Urine: Bence-Jones λ (263.2mg/dl, 7369.6 mg/24h)
- Urine proteins: 379.3 mg/dl (0-10), 10,620 mg/24h (28-141)
- Immunophenotype: 100% PC with aberrant phenotype: CD138+/CD38+/CD19-CD45/CD56-
- Bone marrow aspirate: 63% PC; MRI scan: severe diffuse infiltration pattern of bone marrow.

Conclusion: Interest of this case lies in its rarity, 0.5-1.5 new cases/year/million inhabitants. LCP is classified in primary or secondary (progression of preexisting MM). Both are biologically and clinically different from MM and have poorer prognosis and shorter survival.

We want to stand out the role of the laboratory in the blood-smea review to point immediately the diagnosis.

Abstract number 0214

Thalassemia major phenotype caused by homozygosis for Hb Zurich-Albisrieden [alpha59(E8)GLY-->ARG (alpha2)]

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Hb Zurich-Albisrieden is a highly unstable alpha-chain variant in which glycine was replaced by arginine at position 59 (HBA2:c.187G>C). It cannot be detected at the protein level and a few simple and compound heterozygotes (αZA/αα and --/αZA, respectively) are described so far in Switzerland and China. We describe here a case of homozygosis for Hb Zurich-Albisrieden: a 15-month-old Brazilian boy of European descent with severe congenital hemolytic anemia concomitant to ineffective erythropoiesis, hepatosplenomegaly and blood transfusion dependence, who is being prepared for bone marrow transplantation. His parents (first cousins) and nine siblings are clinically normal, but the parents have already lost two babies. Hb was analysed by electrophoresis at alkaline and acidic pHs, isoelectricfocusing and cation-exchange and reversed-phase HPLC. For molecular analyses, the α-globin genes were initially screened by multiplex-gap-PCR for the common α-thalassemia deletions and restriction enzyme for the non-deletional mutations. Rare deletions were then investigated in both, alpha and beta-globin clusters (MLPA). As no abnormalities were found, the alpha and beta-globin genes were sequenced. The only alteration detected was the GGC→CGC substitution at codon 59 of the alpha2-globin gene, in homozygosis. The patient’s parents and six siblings are carriers. To our knowledge, this is the first description of homozygosis for Hb Zurich-Albisrieden. The previously reported cases of heterozygotes presented with mild erythrocytosis, microcytosis and hypochromia, and the compound heterozygotes with Hb H disease. Despite the presence of two normal alpha-genes, probably the amount of hyperunstable hemoglobin formed in this case is responsible for the severe clinical picture presented by the patient.

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Abstract number 0279

**Gamma-hydroxybutiric acid (GHB) intoxication - a case report**

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Gamma-hydroxybutiric acid (GHB) has become a common drug of abuse worldwide. Hereby we report, according to our knowledge, the first case of GHB intoxication in Croatia. A 33-year old man was admitted to the Emergency Department (ED) due to unknown intoxication. The patient was found in an aggressive state with hallucinations. Shortly upon arriving to the ED, he experienced a cardiopulmonary arrest and was transferred to the Intensive Care Unit. Among other laboratory tests, drug screening was performed (Syva RapidTest d.a.u. 10; Siemens). Confirmation analysis were performed using gas chromatography-mass spectrometry (GCMS-QP2010 Ultra; Shimadzu) after acetonitrile precipitation and BSTFA/1% derivatization.

Laboratory investigation on the day of admission revealed high and rapidly increasing creatine kinase, creatinine and CRP, respiratory acidosi and hyperglycemia. Urinalysis showed gross hematuria. Patient’s blood and urine ethanol levels were 0.00 g/L. Urine toxicology screen was positive for benzodiazepines, cannabinoids and amphetamines which was confirmed by a GCMS method after a conventional sample preparation using liquid-liquid extraction. The presence of GHB was confirmed only after acetonitrile precipitation and BSTFA silylation. Throughout the period of hospitalization the patient experienced delirium, cognitive disturbances, severe agitation and muscle spasms and did not respond to benzodiazepines therapy.

The presented case illustrates the severe consequences of GHB intoxication. Psychotropic effects may be explained by GHB transformation into the inhibitory neurotransmitter gamma-aminobutyric acid (GABA). Supportive care is the key to management. Since GHB analysis is omitted by routine toxicological procedures, its intoxication is difficult to diagnose and may lead to life-threatening complications.

Abstract number 0331

**Positive screening for tricyclic antidepressants. Is it true? Case study**

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Introduction: Antidepressants are widely used for treating depression, as well as benzodiazepines. Although very effective tricyclic antidepressants are also associated with high rate of mortality. The aim of this study was to present a clinical case to demonstrate toxicology screen and serum tricyclic antidepressants immunoassays limitations to confirm the diagnosis of poisoning when in doubt.

Case presentation: A 70 years old female patient with previous history of hypothyroidism and depression was brought to the Emergency Department because of coma and suspicion of antidepressants and/or benzodiazepines overdose. At home patient was polimedicated with bromazepam, lorazepam, diazepam, valproic acid, venlafaxine, duloxetine, clomipramine and trihexyphenidyl.

On admission to the emergency room she had respiratory depression and coma. Analytical study documented severe hypernatremia (164 mmol/L), hypercloremia, nonoliguric acute renal failure (creatinine 2.16 mg/dL, urea 167 mg/dL). CNS infection was excluded as well as status epilepticus syndrome. Urine screening test was positive for benzodiazepines and tricyclics antidepressants. Despite very high concentration of tricyclic antidepressants in the serum by immunoassay (3923 ng/mL) no severe symptoms of cardiotoxicity were found. Posterior benzodiazepines and antidepressant measurements in serum by Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS) showed therapeutic levels for several drugs administered to the patient namely desmethylvenlafaxine, clomipramine, desmethylclomipramine.

In this case cross-reactivity of tricyclic antidepressants immunoassays gave false positive results.

Conclusion: Nonspecific toxicology screen are recommended only if the clinical staff fully understands the specificity limitations of this assays.

LC-MS/MS can be useful in confirmation and evaluation of potential toxicity of several drugs.

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**4th Joint EFLM-UEMS Congress**

**Friday, September 23rd**

**Posters**

**Dyslipidemia: New clinical concepts and diagnostic tools**

**Abstract number 0031**

**Clinical case: acute pancreatitis and lipemia**

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Introduction: Although rare, Lipemia (L) is a source of laboratory (Lb) errors. Macroscopically, sample shows milky aspect. Lipemic index (LI) enables to accurately detect L grade. L interferes with tests which use transmission of light of their measurement system. This interference is due to three distinct mechanisms: light scattering, increasing non-aqueous phase and effects of partition between polar and non-polar phases. Clinical Case: Female, 41 years-old, morbid obesity, HIV+, under KIV (abacavir+ lamivudine) +ATZ/r (atazanavir+ ritonavir), went to emergency due to upper abdominal pain that radiates into the back. Hemodynamically stable. CT: “slight edema next to the pancreatic tail”. Analytically: lactescent serum (LI: 4+; 4814 mg/dL), not allowing serum triglycerides (Tg) and amylase quantification. Urinary amylase: 1274 U/L. Hospitalized with acute pancreatitis (AP) secondary to hypertriglycerideremia due “probable” to antiretroviral (atazanavir). Plasmapheresis was considered but cancelled when Tg quantified (1500 mg/dL). Good response to implemented measures, always with a profile descending Tg and cholesterol.

Conclusions: -After hemolysis, lipemia is the most frequent endogenous interference, influencing laboratory test results. Even though rare, acute pancreatitis secondary to antiretrovirals must be considered in the differential diagnosis of HIV+ patients. Fundamental clinical and laboratory interface.

Bibliographic References:
Abstract number 0072

Progressive cardiovascular disease due to compound heterozygous familial hypercholesterolaemia despite medication, low-density lipoprotein apheresis and liver transplantation

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Homozygous familial hypercholesterolaemia (HoFH) is a rare life-threatening condition characterised by markedly elevated circulating levels of low-density lipoprotein cholesterol (LDL-C) and accelerated, premature atherosclerotic cardiovascular disease. HoFH patients with the same mutation on each allele are ‘simple homozygotes’, those with different mutations from within the same gene are ‘compound heterozygotes’. This report describes a woman with clinical HoFH and compound heterozygosity presenting at age seven years with xanthomata, aortic stenosis and serum cholesterol of 18 mmol/L.

She is heterozygous for two mutations in the LDL-receptor gene: a duplication in exon 4, from her mother, and a single base substitution in exon 10, from her father.

She was treated from 1988 onwards with lipid regulating drugs and LDL apheresis for ten years before requiring liver and heart transplants at age 17 years. Despite these treatments she required a second liver transplant and coronary artery stents at age 30 years.

This case illustrates the difficulties of preventing vascular complications in patients with severe familial hypercholesterolaemia. Lipoprotein apheresis to remove LDL particles did not prevent the development of heart failure. Although liver transplantation did initially allow effective cholesterol lowering, transplant rejection allowed further atherosclerosis to develop. The clinical phenotype of HoFH in this patient is caused by two different LDL receptor mutations.

This report highlights the need for early identification of HoFH, prompt referral to a medical specialist and early initiation of appropriate treatment.

Abstract number 0074

Genome sequencing in a patient with severe gestational hypertriglyceridaemia and follow up treatment over twenty years

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We describe the case of a Chinese woman with lipoprotein lipase (LPL) deficiency treated with lipid regulating medications during a pregnancy at the age of 31 years, and her subsequent management with other lipid regulating drugs over the following 20 years.

A blood sample was screened by DNA sequencing for changes within the LPL and APOA5 genes, and for large deletions/duplications within the LPL gene. Results show that she is heterozygous for LPL c.568G>A; p.Glu190Lys, homozygous for APOA5 c.-3A>G and heterozygous for APOA5 c.553G>T; p.Gly185Cys. The two heterozygous mutations are considered to be pathogenic variants. The homozygous mutation is consider to be a functional polymorphism which may play a role in plasma triglyceride levels.

The coexistence of LPL gene and ApoA2 gene mutations together with gestational diabetes and ApoE2 heterozygosity resulted in marked hypertriglyceridemia and acute pancreatitis during her fourth pregnancy. Treatment of the severe gestational hypertriglyceridaemia with omega-3 fatty acids was effective during the pregnancy before the introduction of colestyramine which allowed further increases in triglycerides. Following the pregnancy, fibrates and statins were initially effective in managing the hyperlipidaemia. At twenty years after pregnancy she developed type 2 diabetes and combination therapy with high doses of statin, fibrate and omega-3 fatty acids was required. Gene replacement therapy for LPL deficiency provides hope as a future therapy for this rare serious condition.

Our findings indicate that knowledge of both genome findings and phenotypic features may guide the clinical management of a rare inherited disease.
Abstract number 0322

**Pseudo-hypocholesterolemia following the extensive consumption of ascorbic-acid in the instance of terminal renal insufficiency. Case report.**

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During a routine control it was impossible to determine the total serum cholesterol concentration according to the common CHOD-PAP method. On repeating the measurement 3 days later an increase in concentration was apparent. However, there was an unexpected raise of the cholesterol concentration after storage of the material for several days at refrigerator temperature, permitting the assumption that during this time, test-interfering substances in the serum sample had been degrading.

After examining the medication which the patient had consumed and detailed questioning regarding eating habits, it was deducted that the cause of the described phenomenon was ascorbic acid (vitamin C), with which the patient orally supplemented her daily intake (3 x 1g/day).

Vitamin C is easily dissolved in water; excessive quantities are exhausted through the kidney as urine and therefore the rarity of hypervitaminosis. In the cause of renal insufficiency, ascorbic acid is retained for considerably longer period in the blood circulation. Because it is easily oxidized, it appears anti-oxidative and has the effect of a radical arrestor: this characteristic influences the CHOD-PAP method.

Conclusion: Vitamin C is produced world-wide in massive quantity and diversely used and particularly so since L. Pauling, as a dietary supplement. The ascorbic acid concentration in the blood has probably consequences for in vitro cholesterol measurement; in particular in patients with renal insufficiency and following vitamin C-infusion.

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Posters

**New diagnostic tools in infectious diseases**

Abstract number 0001

**Evaluation of care, maintenance and user practices of medical laboratory equipment and their impact on the effectiveness of laboratory operations at four central hospitals in Malawi**

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Background: Laboratory services are essential to supporting and improving health service delivery and are dependent upon the availability of functional equipment. Medical equipment is indispensable for the prevention, diagnosis, treatment, and management of all diseases. Functional equipment requires maintenance, spare parts, reagent supplies and proper use. There has been lack of preventive maintenance, user training and proper care of equipment in hospitals in developing countries.

Methods: A cross sectional study design was used in which 42 questionnaires were administered to professional and certified laboratory personnel at four central hospital laboratories in Malawi who had reported to work on the day of data collection.

Results: More equipment inventories are kept in electronic form. Preventive maintenance is performed. On-job user and care trainings are conducted. External repair services are reliable but expensive and take long time to do repairs. All donated equipment come mostly with all the required resources. Purchase and operation of equipment are heavily funded by donors.

Conclusions: This study has found that there are improvements in care, use and maintenance of laboratory equipment in central laboratories due to heavy donor support and implementation of SLMTA/SLIPTA programs. A lot of equipment is on service contracts with improved availability of reagents, repair technicians, spare parts and user training. This is in sharp contrast to what used to be the case in the past. With continued donor support equipment management will continue to improve which will directly improve access to decent diagnostic services in Malawi.
Abstract number 0063

Rapid detection of varicella zoster virus by lateral flow immunoassay (LFIA)

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Background: Varicella zoster virus (VZV) is one of Herpes viruses for humans. First infection by VZV causes varicella (chicken pox). Even when clinical symptoms of chicken pox have resolved, VZV is dormant in ganglion of the infected person. VZV reactivates within immune compromised person, has kept VZV in ganglion. In many cases, it is difficult to discriminate between clinical symptoms for VZV infection and that for Herpes simplex virus (HSV) infection. Recently, VZV / HSV identification takes on a growing importance in a method of medical treatment by development of selective antiviral drugs for VZV or HSV infection.

Method: We have developed an antigen detection test kit based on lateral flow immunoassay using mouse anti-VZV Glycoprotein E (VZV gE) monoclonal antibody. The sensitivity and cross-reactivity were confirmed recombinant VZV gE, inactivated VZV, inactivated HSV-1 and HSV-2 diluted with running buffer containing surfactant. The intensity of color on detection line, determination time 5 min, was measured by densitometer.

Results: Our results indicates that the developed of LFIA for VZV don’t have cross-reactivity for HSV. It can be detected VZV within 5 minutes and limit of detection for the LFIA is VZV gE 1 ng/mL, inactivated VZV 5 ug/mL.

Abstract number 0073

Alcohol dehydrogenase isoenzymes and aldehyde dehydrogenase activity in the course of hepatitis C

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The hepatocytes destruction is reflected by increased different enzymes activity in the serum. These enzymes include alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH), which exist mainly in the liver and play role in metabolism of many biological substances. In this study we investigated the activity of alcohol dehydrogenase isoenzymes (class I-IV) and the total activity of ALDH in the sera of patients with hepatitis C.

Serum samples were taken from 55 patients (33 males, 22 females 31-75 years) suffering from viral hepatitis C. Clinical diagnosis of illness was made on the basis of serological examinations. Class I and II ADH isoenzymes and ALDH were measured by fluorometric method using specific substrates (4-methoxy-1-naphtaldehyde and 6-methoxy-2-naphtaldehyde respectively). Total ADH activity was estimated by the photometric method with p-nitrosodimethylaniline.

We have found that the activity of ADH I was more than twice elevated in viral hepatitis C (3.94 ± 1.73mU/l) in comparison to the control level (1.75 ± 1.33mU/l) (p<0.001). The ADH II activity also significantly higher among patients with hepatitis C (25.54 ± 8.35mU/l) than healthy ones (12.76 ± 6.22mU/l). Activities of both classes of ADH isoenzymes have a good correlation with alanine and aspartate aminotransferase. The increase in total alcohol dehydrogenase activity was not very high but confirmed the elevation of class I and II isoenzyme activity.

The increase of the activity of class I and II ADH in the sera of patients with HCV infection seems to be caused by release of these isoenzymes from damaged hepatocytes.

Abstract number 0118

Monitoring of novel urinary protein markers in sepsis

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Introduction: Diagnosis and monitoring of sepsis is challenging even nowadays. Although proteinuria is a well-known phenomenon in sepsis, the clinical usefulness of urinary proteins has not been explored yet. Our aim was to monitor different urinary proteins as diagnostic and severity markers of the septic process. We investigated the acute phase protein orosomucoid, the cell component actin and cystatin-c in urine.
Materials and methods: Urinary orosomucoid (u-ORM; Dako) and cystatin-c (u-CYSC; DiaSys) were measured by automated immune turbidimetry, while urinary actin (u-ACT) levels were determined by quantitative western blot in urine samples of septic patients (n=35) and in controls (n=53). We referred the concentration data to urinary creatinine levels. Our results were presented as medians.

Results: 240-fold higher u-ORM values were found in sepsis than in controls (19.31 vs 0.08 mg/mmol, p<0.001) and additional extreme u-ORM levels were measured in dialyzed septic patients. We could not detect u-ACT in urine samples of controls in contrast to sepsis. Significantly elevated u-ACT was found in samples of patients with sepsis-related acute kidney injury (AKI) compared to non-AKI patients (1.67 vs 1.05 ng/mmol, p<0.05). U-CYSC levels were higher in sepsis compared to controls, as well (0.234 vs 0.007 mg/mmol, p<0.005).

Conclusions: The early and relevant increase of u-ORM suggests that it might be a promising novel diagnostic marker of sepsis. U-ACT concentrations might indicate acute kidney injury. U-CYSC is a reliable marker of tubular damage. These novel parameters provide useful information on the septic process and could help clinicians in rapid decision making.

Abstract number 0166

Evidence of potential occult HBV infection in selected Italian populations

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Study aims: Mass vaccination against hepatitis B virus (HBV) started in Italy in 1991. Individuals born after 1979 were not included in the vaccination campaign and represent a potential reservoir for this infection. We evaluated the prevalence of HBV infection during routine screening.

Methods: We included all individuals that resulted anti-HBc positive (Abbott ARCHITECT) over one year. Samples were tested for other HBV markers (HBsAg, anti-HBs, HBeAg, anti-HBe) by the same system. Anti-HBc and anti-HBe were assayed also Liaison (Diasorin). On selected individuals, HBV-DNA and/or HCV-RNA were tested for by real-time PCR (Roche).

Results: Out of 2,454 individuals screened, 91 (3.7%) resulted positive for anti-HBc (59/60 confirmed by Liaison); 16 of them were tested before bone marrow donation and 19 for assisted reproduction. The mean age was 58.4±14.8 years (median: 60), females were older and six subjects (6.6%) belonged to vaccinated age cohorts. HBsAg was positive in 5 HBV+ subjects (5.5%), anti-HBs in 58 (63.7%). None was positive for HBeAg; anti-HBe was detectable in 59 subjects (64.8%) by ARCHITECT and Liaison identified 14 additional reactivities. Four subjects were coinfected by HCV and HBV-DNA was detectable at low levels (68-1,000 IU/mL) in 2/9 (22.2%) individuals.

Conclusions: The positivity for HBV serological markers also in subjects belonging to vaccinated cohorts and the evidence of low levels of HBV-DNA in subjects negative for HBsAg, confirm that an occult HBV infection (HBV) may be a quite frequent finding by routine testing. The implications for bone marrow donation and assisted reproduction shall be considered.

Abstract number 0183

The impact of presepsin in prognosis: measurement in intraabdominal sepsis

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Introduction: Sepsis, a leading cause of death in critically ill patients, is the result of complex interactions between the infecting organism and the host responses that influence clinical outcomes. The early diagnosis of sepsis plays central role in patient management. First evidence suggested that presepsin may serve as a diagnostic and also as prognostic marker. The aim of this study was to evaluate the early prognostic value of presepsin compared with Acute Physiology and Chronic Health Evaluation II (APACHE II) score in septic patients.

Methods: We determined presepsin and APACHE II score in patients with sepsis in different sepsis conditions. EDTA plasma samples were collected to examine presepsin with PATHFAST Presepsin test, Mitsubishi Chemicals. The Acute Physiology and Chronic Health Evaluation (APACHE) II score was used as an index of severity of illness. Score is calculated based on the results of the first day.

Results: Patients were stratified into three groups: sepsis, severe sepsis and septic shock. The mean values for APACHE II score in different stages were 10.1, 19.2 and 24.8 with statistically significant differences between groups. Early presepsin values (day first) were especially high in severe sepsis and sepsis shock and there was a significantly positive correlation between the APACHE II scores as a severity index and presepsin.

Conclusion: In fact early presepsin values significantly correlated with poor outcomes for patients with severe stages of sepsis. These suggest that the presepsin might serve as an valuable prognostic biomarker for evaluation of prognosis in septic patients.
Abstract number 0273

Frequency of positive test results of IgA antibodies to Bordetella pertussis toxin in adults

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Aim of the study: Amount of adults diagnosed with Pertussis increased. The aim of the study was to determine the frequency of positive test results of immunoglobule A antibodies (IgA) to Bordetella pertussis (BP) toxin and possibility of occurrence of Pertussis in adult patients.

Material and methods: 2377 adult patients within age range 18-107 (1646 female, 731 male) were tested with IgA ELISA test (Euroimmun) in laboratory of Diagnostyka Company. Four age groups were considered: 18-30 year [yr] (1), 31-40 [yr] (2), 41-50 [yr] (3), above the 50 [yr] (4). 49 patients were tested more than once.

Results: From 2377 patients, 2328 were tested only once (TO). The frequency of supposing Pertussis was 28.95% (675 patients) in TO and 28.90%, 26.00%, 37.65%, 26.08% for each 1-4 groups respectively. From 49 monitored patients IgA value have increased four times in second test (4%), remain very high, above the linearity of the test (regardless of the amount of tests performed) in 12 (25%), high in 13 (27%), decreased in 10 (20%) and in normal range in 12 (25%) (Pertussis excluded). Mean age of 37 patients with high level of IgA were 46 years (19-76) but nearly half of the people belonged to the group 4 (16 patients).

Conclusion: The frequency of positive test results of IgA antibodies to Bordetella pertussis (BP) toxin elevated according to age and the most exposed patients were in age above to 40 years. Remedies for that group supposed to be taken into consideration.

Abstract number 0311

Platelet count (PC) and mean platelet volume (MPV) - could they predict severity of sepsis?

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Aim: Sepsis is uncontrolled inflammatory response to viral, bacterial or fungal infection. Thrombocytopenia and coagulation abnormalities associated with disseminated intravascular coagulation are common in sepsis. The aim of this study was to estimate whether MPV and PC are in relationship with procalcitonin (PCT) and therefore could be prognostic markers for severity of sepsis.

Methods: Our study included 55 patients with sepsis; median age was 72 (20-96) years, 18/55 were women, and 20 healthy controls; PCT <0,05 ng/ml, median age was 62 (24-90) years and 6/20 were women. We measured PC and MPV on Cell Dyn 1800, Abbott Diagnostic, USA and PCT was measured using the enzyme-linked fluorescent immunoassay, VIDASR BRAHMS PCTTM, BioMerieux, France.

Results: Compared with control group PC was lower (p=0,0001) and MPV was higher (p=0,0007) in patients with sepsis. There was not correlation between MPV and PCT (r = -0,07504; p = 0,5861; R2= 0,0056) nor between PC and PCT (r = -0,090; p = 0,5153; R2= 0,2655) in patients with sepsis.

Conclusion: In this study we concluded that MPV is higher and PC is lower in patients with sepsis, but MPV and PC are not in correlation with PCT. Therefore we cannot predict severity of sepsis by measuring only MPV or PC.
Methods: A 2 U/mL IgA-tTG cutoff was established using p95 of 3/438 patients without celiac disease. A and G anti-gliadin antibodies levels were performed in samples with IgA-tTG between 2 and 10 U/mL (grey zone). Patients with high levels of IgA or low levels were removed from the study as well as patients under two years old. Statistical analysis was performed with STATA 13.

Results: A total of 28 (2.5%) patients presented results in IgA-tTG levels grey zone. 60.7% of these patients were negative for anti-gliadin and the others were positive (39.3%). Using usual cutoff (7 U/mL), 22 uncertain results were obtained and 7 of them were positive (31.8%) and 15 were negative (68.2%). There were 179% of patients with uncertain IgA-tTG values with positive anti-gliadin antibodies, which had not been detected with usual cutoff. This fact was important in children under ten years old. There were 6 children and 5 adolescents between ten and twenty years old. Related to sex, there were 4 women and 7 men.

Conclusion: In conclusion, this study point out the importance of changing the cutoff for uncertain values in celiac disease screening test, overall in patients under 18 years old and in women.

Abstract number 0117

Multicenter evaluation of the new IMMULITE 2000 XPi TSI assay for the diagnosis of Graves’ disease

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Introduction In Graves’ disease (GD), thyroid-stimulating immunoglobulins (TSI) bind to the TSH receptor (TSHR) and mimic TSH stimulation, leading to hyperthyroidism. The large extracellular domain of the TSHR presents epitopes for a variety of autoantibodies, including thyroid-blocking immunoglobulins (TBI). TSHR autoantibody (TRAb) assays do not distinguish between TSI and TBI. The IMMULITE 2000 XPi TSI assay utilizes recombinant human TSH receptors (hTSHR) for the specific detection of thyroid-stimulating autoantibodies. Our study objective was to evaluate the analytical and clinical performance of the fully automated TSI assay. Method A multicenter validation of the IMMULITE 2000 TSI assay was performed measuring sera from healthy controls (n = 40), patients with GD (n =110), Hashimoto’s disease (n=27), thyroid carcinoma (n=17), morbus De Quervain (n=21) and toxic multinodular struma (n=5). Analytical and clinical performance was evaluated. Results The analytical performance showed a high inter-laboratory correlation (R2 > 0.98, n = 40), a within run %CV of 3.9% and 3.3% for serum samples with 1.1 IU/L and 25.4 IU/L respectively and a repeatability %CV during an on-board reagens period of ninety days which varied from 3.9 to 6.1% across the assay range. Analysis of the clinical performance for GD at 0.55 IU/L cutoff showed a sensitivity of 89% and 90% specificity, compared to 95% sensitivity and 76% specificity for a conventional TSH-R antibody assay. Conclusion The IMMULITE 2000 XPi TSI assay is a robust, sensitive and specific quantitative immunoassay for the specific detection of TSI in the routine diagnosis and assessment of GD patients.

Abstract number 0237

Automation in indirect immunofluorescence testing and its impact on work organization in autoimmunology laboratory

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Aim of the study: Automatization of indirect immunofluorescence (IIF) analysis leads to the standardization of technique for the determination of autoantibodies. The aim of the study was to indicate the advantages resulting from implementation of the EUROIMMUN automated system.

Materials and Methods: Automated system was implanted in autoimmunology laboratory of Diagnostyka Company in Warsaw. The system consists of: EuroLabOffice Software (ELO), Sprinter XL (automated instrument for incubation immunofluorescence testing) and BIOCHIP Technology (cover glasses coated with biological substrates).

Results: The connection of ELO and Sprinter XL resulted in: automatic sample identification through scanning, automatic dilution and dispensing of samples to the incubation, efficient washing of slides and their evaluation through a microscope specifically tailored to the requirements of IIF. Optimization of work organization was achieved by generating incubation protocol worklist, automated sample dilution, bidirectional data transmission between laboratory system (LIS) and the analyzers. It led to increased throughput workflow by 60%. The time required to perform for the incubation was reduced by 40%. Estimated time between receiving the samples and distribution of results was also reduced by 30%. Modern washing technology for brilliant fluorescence, liquid level detection and precise pipetting provides quality and security. Using BIOCHIPs coated with different substrates on a single reaction field allows to identify many different antibodies in one incubation. Conclusions: Implementation of the automated Euroimmun system in assures standardization and simplification of the IIF analyses performed and an opportunity to manage a large researches and to report the results faster.
Nitric oxide concentration and nitric oxide synthase activity in patients with systemic lupus erythematosus

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Nitric oxide, as the smallest ubiquitous cell signalling mediator, has an important role in immune regulation and pathogenesis of SLE. Pro-inflammatory effects involve vasodilatation, edema, cytotoxicity and mediation in cytokine-dependent processes which can possibly induce tissue destruction.

This paper investigated the status of NO system, NO concentration (Cayman Chemical test) and i-NOS activity (Bioxytech Nitric Oxidase Synthase Assay kit-Oxis International) in the blood plasma of 55 SLE patients. The patients, in acute exacerbation disease phase were divided into four groups: skin (S-SLE), neurologic (N-SLE), joint (J-SLE) and vascular (V-SLE) disease manifestation, while 20 healthy volunteers, comprised the control group.

Highly elevated values of NO were obtained in N-SLE (43.26 ± 2.91 μmol/l), J-SLE (42.97 ± 5.64 μmol/l) and V-SLE (42.48 ± 6.36 μmol/l) related to control values (20.94 ± 4.64 μmol/l) for P < 0.01. The highest activity of i-NOS was registered in V-SLE (M = 1.40 nmol/ml) related to controls (M = 0.72 nmol/ml) (P < 0.001), with lower statistical significance in S-SLE (M = 1.27 nmol/ml, P < 0.01) and, finally, in J-SLE (M = 1.18 nmol/ml, P < 0.05).

Excessive NO production can be followed by the induction of i-NOS expression. The degree of induction varies in different clinical disease manifestations, depending on the degree of synergy between bacterial products and cytokines. Long-term exposure to NO may induce irreversible damage to mitochondria in the cells, commonly mediated by reactive oxygen radicals. Selective i-NOS inhibitors may reduce excessive NO production, and they have been recommended recently in SLE treatment.
Abstract number 0194

Nutritional evaluation by means of biochemical parameters in elderly patients admitted to hospital

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One's nutritional status has an important effect on the progression of any disease; this is even more important in elderly patients. A quick assessment can improve patients' status and shorten their hospital stay. Other ways of evaluating nutritional status are costly and subject to variability amongst individuals. The Medical Laboratory can correctly perform a nutritional evaluation in a quick and efficient manner.

Objective: Validate the utility of the CONUT nutritional evaluation method performed by the Medical Laboratory on all elderly patients at Hospital Serranía de Málaga.

Materials and Methods: The CONUT method for nutritional evaluation was used. This method is based on determining undernutrition according to levels of serum albumin, total lymphocyte count and total cholesterol for all patients admitted to Hospital Serranía de Málaga in 2015. Samples were analysed by the Medical Laboratory. The frequency of nutritional risk was calculated analysed alongside data on patients' sex, age and the hospital department to which they were admitted.

Results: 1842 patients were studied. 53.2% were not undernourished, 31.1% were moderately undernourished and 15.7% were severely undernourished. The mean age of patients was 64.09 years. 50.2% were women and 49.8% were men. 145 patients were severely undernourished, of which 55.1% were men and 44.8% were women, mean age was 68.2 years and the main department to which they were admitted was Internal Medicine, 88.2%.

Conclusion: The nutritional evaluation performed by the Medical Laboratory is an effective tool to evaluate nutritional status and take corrective measures.

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Posters

Clinical applications of genome sequencing

Abstract number 0114

Association of GSTM1 and GSTT1 gene deletions polymorphisms and framingham risk-score (FRS) in subjects who lives near central gas facility

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Aim: To examine the influence of exposure to central gas station (CGS) and presence of GSTM1 & GSTT1 genetic polymorphisms on FRS, serum lipid concentrations and anthropological factors.

Material and methods: Case-control study included 252 Croatian subjects of group exposed to CGS in Molve district and 148 unexposed subjects (Rasinja district). Fasting serum lipids concentrations were determined with standard methods on COBAS Integra 400 (Roche, Indianapolis, USA). Genetic polymorphisms were genotyped with multiplex PCR, (Roche Diagnostics, Mannheim, Germany). Cardiovascular risk assessment was performed by using FRS calculation. Statistical analysis (MedCalc software version 10.20.0, Mariakerke, Belgium) included Wilkinson test, logistic regression, t-test or Man-Whitney and MANCOVA. The level of significance was set at 0.05. An alpha correction to account for MANCOVA was performed according Bonferroni.

Results: Logistic regression show that exposure and GST polymorphisms cannot be considered as a risk confounders for increased FRS. Total cholesterol and triglyceride concentrations were significantly higher in exposed subjects with GSTT1-1 allele. BMI were significantly higher in exposed subjects with GSTM1-0 allele (P=0.050). MANCOVA analysis which included total HDL- and LDL- cholesterol, glucose and BMI with covariates sex, age, diabetes mellitus, hypertension and smoking status, showed that there is a significant difference between GSTT1-0 and GSTT1-1 allele carriers of exposed group (Wilkinson test=0.91, p=0.001, F=3.98).

Conclusion: There were no interaction of GSTM1 & GSTT1 genetic polymorphisms and exposure to PAHs in increasing of CAD risk, but both polymorphisms might be associated with changes of lipid profile in subjects exposed to gas facility.
Abstract number 0170

The role of the inheritance in the open angle glaucoma primitive

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Introduction: Glaucoma is the leading cause of irreversible vision loss in the world. Primary open angle glaucoma (POAG) is the most common form of glaucoma with a prevalence of 2.8%. The role of heredity in the POAG was raised long ago. The main objective is the identification of subjects at a preclinical stage.

Materials and methods: Genetic risk factors are known to contribute to POAG when those affected are first-degree relatives and are 3-9 times more likely to develop the disease. Identification of genetic risk factors could greatly improve the detection and treatment of POAG. Patients who are aware of the signs and glaucoma who suspect they may have the disease can occur earlier.

Result: The identification of a gene associated with glaucoma (MOY / TIGR) is also likely to improve our understanding of physio disease pathogenesis and therapeutic developments. The authors sought to determine whether the glaucoma patient with a family history of the disease were younger and showed fewer signs of glaucomatous optic neuropathy at diagnosis compared to glaucoma patients without a family history of the disease.

Conclusion: POAG is a major public health problem, requiring systematic mass screening, particularly in the population over 40 years. This screening is based on the measurement of intraocular pressure and analysis of the optic disc by the fundus and identification of the gene MYO allows today to consider the direct screening of individuals genetically predisposed to develop disease. Further studies are needed to unravel the increased familial risk genetic components.

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Point-of-care testing: Methodology and quality

Abstract number 0042

Assessment of erythrocyte sedimentation rate in POCT

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Background: ESR gives data useful for following the course of disease, efficiency of therapy and prognosis of disease. The aim of the study was to evaluate analytical reliability, precision and accuracy for determination of ESR using comparative examination of automated Alifax Roller 20 MC analyzer and Westergren method with attempt to characterize Alifax methods as correct and precise for uses in POCT diagnostics.

Methods: The studied subjects were 104 students, during preventive systematic examination, mean age of 23. 14 ± 2. 04 years, both sexes (33 men and 71 women).

Results. Results showed serial precision for Roller 20 MC (within-run imprecision CV%, n=104) was 1,20 %. Sensitivity of Alifax method is 98,7 %, and specificity is 95%. Correlation of two series with Alifax method showed good correlation (r=0,957), analyzer had a good reproducibility. Accuracy of results on Roller 20 MC was 96,1%. Acquired results showed very good correlation between two methods Alifax vs. WGSE (r=0,960). Positive correlation was established between Alifax method with parameters of CBC and parameters of inflammation CRP (r=0,671) and FIB (r=0,688) (p < 0.01) in non-specific diagnosis, and fit into diagnosis and clinical status and had significantly adventage for better diferential diagnostics of different diseases and pathological states in young people.

Conclusions: These data suggests that Alifax methods are reliable and suitable as well as very specific, have high sensitivity, gives quick results, ease of use, may be used in emergency laboratories for rapid diagnosis of urgent clinical states as POCT.
Abstract number 0090

Analytical validation of four POC glucometers

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POC devices for measuring blood glucose are widely used in hospital settings as a convenient way to rapidly manage patient therapy. However, selection of the glucometers for hospital use must be carefully provided since numerous glucose meters are available on the market rarely achieve analytical performance goals given by ADA or IFCC.

The aim was to investigate analytical performance of four glucometers versus reference laboratory method.

Glucometers of four manufacturers [FreeStyle Optimum Neo (Abbott Diabetes Care Inc., Almeda, CA, USA), Bionime GM550 (Bionime GmbH, Switzerland), On-Call Vivid (ACON Laboratories Inc., San Diego, CA, USA) and Element (Infopia Co Ltd, Korea)] were validated. Laboratory glucose was measured with the hexokinase method on Architect c2000 (Abbott Laboratories, Abbott Park, IL, USA). Venous blood was drawn from 30 T2DM patients and healthy volunteers in Li-heparin vacutainers (Greiner Bio-One, Kramsmünster, Austria). Capillary blood glucose was determined on all glucometers for each patient after venous blood sampling. Venous glucose was determined immediately after venipuncture.

Results of imprecision were: FreeStyle 6.7%; Vivid 4.4%; Element 4.0%; GM550 3.3%. Relative mean biases between glucometer and reference method were: Freestyle 9.1%; GM550 15%; Vivid 9.1%; Element 12.2%. Passing-Bablok regression analysis have shown systematic shift for Element (\(y = 0.751553 (0.03500-1.7000) + 0.965839 (0.8333-1.1000)\)) and systematic and proportional difference for GM550 (\(y = 1.170588 (0.9122-1.3955) + 0.764706 (0.7273-0.8049)\)). Error grid analysis showed 100% results in A zone for FreeStyle and Vivid while Element and GM550 showed 81% and 94% results in Zone A, respectively.

Results of the study showed satisfactory analytical performance for only two devices, FreeStyle and Vivid.

Abstract number 0109

Whole blood creatine kinase (CK) determination with the reflotron system: interest in the follow up of international rugby players

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CK is regularly assayed in blood tests as a marker of skeletal muscle. Clinically CK is analyzed in muscle diseases but CK testing may be used also to follow intensive sport activities in particular rugby. According to sport field conditions it is difficult to obtain venous blood. Only a point of care system like Reflotron may be used easily. Capillary whole blood can be drawn with skin puncture from rugby players after intensive training or matches on decentralized testing sites. We report here on the evaluation of Reflotron CK in sport field conditions with a comparison serum method on CobasBio in clinical laboratory. Our study was conducted in 2014 with 25 French national team players during a 10 days intensive training period including an international match. We examined 150 whole blood and serum samples of which 120 had an elevated CK activity (> 200 U/l). For samples with CK activity > 1500 U/l on CobasBio and > 1900 U/l on Reflotron a dilution was realized. Our results show that Reflotron CK gives values identical to the comparison method irrespective of the source of blood samples (Pearson correlation: lower CK: 0.93; upper CK: 0.98). Reflotron CK allows rapid and reliable measurement of whole blood CK which needs only skin puncture. Reflotron CK may be considered as a suitable alternative for uncommon decentralized testing sites. This method is now routinely used by professional rugby teams. Other trials are carried out in Judo for the next Olympic Games.

Abstract number 0146

Gem Premier 5000 (Werfen): analyzer evaluation, risk assessment and practicability

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Aim of the study: To evaluate the Gem Premier 5000 (Werfen) a new portable blood gas/CO-Oximeter/hematocrit/electrolytes/glucose/lactate/bilirubin analyzer intended for stat laboratory or point-of-care (POC). The only reagent required is a disposable cartridge which contains all materials required to perform analytical testing including quality controls (Intelligent Quality Management 2, IQM2).
Methods: Evaluation was made according to the French Society of Clinical Biology: within-run precision and between-day precision at 3 levels, accuracy with external quality assessment, comparison of methods with 250 routine patient samples, reportable ranges, and endogenous/exogenous interferences for CO-Oximetry. Comparisons analyzers were ABL835 (Radiometer) and DXC8000 (Beckman-Coulter) for electrolytes/glucose/bilirubin. Risk assessment was done using Ishikawa diagram and FMECA method. Practicability was evaluated in the laboratory and by POC users. Results: Within-run and between-day imprecision gave conform CV values for all parameters (except for PO2 at 25mmHg - no clinical incidence). Accuracy was satisfactory and linearity was within the expected ranges given by Werfen. Method comparisons showed close agreement (0.95 < r < 1.00). No interferences on CO-Oximetry parameters were found. New features of the GEM 5000 (IQM2 and the IntraSpect check process to identify potential errors) contribute to minimize the risks especially in POC conditions. Users consider the GEM 5000 as a very positive evolution of GEM 4000.

Conclusion: Gem Premier 5000 analytical performances are validated for all parameters with the accuracy and the reliability of traditional systems. This analyzer fulfills the requirements for a POC or a stat laboratory usage and is an improvement in the GEM series.

Abstract number 0151

Hemoglobin determination comparing a gas analyzer to a hematology analyzer

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Objectives: Our purpose is to compare hemoglobin (Hb) levels obtained by the POCT system (gas analyzer GEM Premier 6000, Werfen group, Izasa) with the levels obtained using a hematology analyzer (DXH BeckmanCoulter).

Material and methods: A total of 181 samples from patients admitted in the ICU were analyzed. A lithium heparinized syringe for blood analyses and an EDTA tube for blood count were obtained at the same time. Both analyzers carry out Hb quantification using the spectrophotometry method. The correlation study was performed using Pearson correlation test. The obtained values were compared using Student’s t-test.

Results: Hb levels quantified using both methods are significantly related with a correlation coefficient of 0.725 (p<0.001). A statistical significance of overestimation of Hb was reach when using the gas analyzer with an average of 0.45 g/dL (p=0.002) compared to the hematology analyzer. Patients were classified into three groups according to Hb levels: 4.5-9 g/dL; 9-13 g/dL and the 13-21.5 g/dL. We studied if there were differences in the level of Hb of each group using both methods. The only group without these significant differences was the one in which we measured 9-13 g/dL Hb levels using the gas analyzer (p=0.31).

Conclusion: In our study, we observed statistically significant correlation between Hb levels determined by both methods in hemoglobin levels between 9-13 g/dL quantified in the gas analyzer. Hb levels lower than 9 g/dL or higher than 13 g/dL quantified using the gas analyzer must be confirmed in the laboratory using a CBC analyzer.

Abstract number 0163

Comparison of two quality control methods of POC glucometers in University Hospital Brno

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Aims: Two external quality control methods recommended by the Czech Society for Clinical Biochemistry were carried out to provide data on analytical performance of hospital point of care (hosPOC) glucometer system AccuChek Inform II (ACII; Roche) counting 100 devices.

Methods:

a) Comparison method (CM): Glycaemie of two patients were measured with each ACII and samples of their capillary blood were sent to and analyzed in the hospital laboratory (Cobas 6000; Roche). The results were compared to values obtained in the laboratory.

b) Reference material method (RMM): The hosPOC supervisors visited departments where they measured reference materials provided by the Czech institute for external control (SEKK) with ACII. The results were compared to the reference values.

Results: The two control methods showed significant difference (F-test) in variation of results for both low or normal (variation ratio=9.15; p<0.001) and increased glycemie (variation ratio=10.6; p<0.002) compared to the hematology analyzer. Patients were classified into three groups according to Hb levels: 4.5-9 g/dL; 9-13 g/dL and the 13-21.5 g/dL. We studied if there were differences in the level of Hb of each group using both methods. The only group without these significant differences was the one in which we measured 9-13 g/dL Hb levels using the gas analyzer (p=0.31).

Conclusions: The analytical performance was as much as four times worse in CM compared to RMM. This was attributed to improper choice of tested patients with contraindicative conditions and bad sampling practice. This may be solved by an improved ACII users training.

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Biomarkers in neurology

Abstract number 0014

Effect of the presence of blood cells in the CSF on the stability of chosen proteins

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The object of the study is to obtain data on the stability of selected proteins, analysed in the cerebrospinal fluid, in relation to stability as a function of time, and to the presence of blood cells. The study includes the following proteins: C-reactive proteins, transferrin, prealbumin, orosomucoid, complement C3, complement C4, NSE, S100B.

Samples were prepared from native cerebrospinal fluid, with concentrations of the blood cells (erythrocytes) at 10/3 ul, 1000/3 ul, 10000/3 ul and 100000/3 ul CSF. These were compared with concentrations in reference samples after 1, 3 and 7 days. Measurements were taken of the concentrations of the proteins in the cerebrospinal fluid. The concentration measurements were carried out using a BN ProSpec Nephelometer and a Cobas 411 analyser.

The results show that the protein concentration is significantly influenced by erythrocyte concentrations greater than 10000/3 ml. Changes take place in the C3, C4 and Transferrins at erythrocyte concentrations of 100000/3 ul.

The results obtained demonstrate a good stability of proteins in the cerebrospinal fluid. This is due to the fact that changes in the concentrations as a result of the number of cells present and the storage time are very unlikely.

Abstract number 0252

Serum elements analysis in Alzheimer’s disease

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Alzheimer’s disease (AD) is the most common cause of dementia, estimated to contribute to about 60–70% of cases and is characterized by a progressive decline of memory and other cognitive abilities. The identification of early biomarkers for diagnosing AD and other forms of dementia are increasingly important. Compelling evidence shows that metal toxicity plays a crucial role in the onset of AD, inducing uncontrolled oxidation and neuroinflammation.

For better understanding the potential role of metal dishomeostasis in the onset and progression of cognitive deficit, we measured by inductively coupled plasma mass spectrometry the serum concentrations of 22 elements in 34 patients with probable AD, 20 with mild cognitive impairment (MCI), 24 with subjective memory complaint (SMC) and 40 healthy subjects (HS). The analysis of variance showed that manganese, iron, copper, zinc, selenium, thallium, antimony, mercury, vanadium and molybdenum changed significantly among the 4 groups. Essential elements, such as manganese, selenium, zinc and iron tended to increase in SMC and progressively decrease in MCI and AD. Toxic elements showed a variable behavior, since vanadium, strontium, tin, arsenic, and uranium tended to increase, while mercury and antimony tended to decrease in AD. A panel composed by six essential elements (manganese, iron, copper, zinc, selenium and calcium) and their respective ratios discriminated AD patients from HS with over 90% accuracy.

Our results suggest that metals contribute to generate a distinctive signature during the progression of AD, and their monitoring might help to detect its preclinical stages.
25-OH vitamin D as a prognostic factor of chemotherapy-induced neuropathy

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Chemotherapy-induced peripheral neuropathy (CIPN) is an adverse effect of Paclitaxel (taxane group), cisplatin, oxaliplatin and vincristine oncology treatment. The incidence of CIPN is around 70% and a CIPN diagnosis is a limiting factor for subsequent oncologic treatment. The aim of the study was to evaluate vitamin D status in a group of patients and to determine its possible use as a predictor of CIPN in the treatment with paclitaxel. Methods: Plasma 25-OH Vitamin D (D2 + D3) levels were analysed by Liaison XL (Diasorin, Italy) in 40 patients. Samples were obtained before paclitaxel treatment. After 12 weeks, CIPN occurrence was observed in 20 cases. Obtained data were analysed by SigmaPlot software (Systat, USA) - Mann-Whitney Rank Sum test. The data are presented as a median (25%; 75%). Test performance - ROC curve was analysed by Analyse It for Microsoft Excel.

Results: Plasma vitamin D in group of patients without CIPN was 43.7 (31.53; 49.63) and in group patients with CIPN 28.5 (23.85; 39.93) nmol/l. P value is 0.004. The area of ROC curve was 0.77, P value 0.0003 and when a cut-off value 35 nmol/l was used, sensitivity and specificity 75% were found.

Conclusion: According to the results of this pilot study, vitamin D could be a possible marker of CIPN prediction. Supported by Ministry of Health Czech Republic - Development and Research Organization- University Hospital Hradec Kralove (MH CZ DRO (UHHK, 00179906).

Abstract number 0338

Levels of acyl-carnitines in serum of patient with or at risk of Alzheimer's disease

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Alzheimer's disease (AD) is a progressive brain disorder that leads to the loss of cognitive functions and behavioral abilities.

Some transitional states between normal aging and AD have been identified, known as mild cognitive impairment (MCI) and subjective memory complaint (SMC). MCI refers to the clinical condition in which persons experience memory loss and/or other cognitive impairments greater than expected, while SMC indicate a report of memory problems without pathological results on neuropsychological tests.

This study aimed to determine the serum levels of free L-carnitine, acetyl-L-carnitine and 34 acyl-L-carnitine in healthy subjects and in patients with or at risk of AD. Twenty-nine patients with probable AD, 18 with MCI, 24 with SMC and 46 healthy subjects were enrolled in the study. The levels of carnitine and acyl-carnitines were measured by tandem mass spectrometry.

The results showed that serum acetyl-L-carnitine and other acyl-L-carnitines (malonyl-, 3-hydroxyisovaleryl-, hexenoyl-, decanoyl-, dodecanoyl-, dodecenoyl-, myristoyl-, tetradecenoyl-, hexadecenoyl-, stearoyl-, oleyl- and linoleyl-L-carnitine) levels decreased along the continuum from healthy to SMC and MCI subjects, up to patients with AD, and that the metabolism of some acyl-carnitines is finely connected among them.

These findings suggest that the serum levels of acetyl-L-carnitine and other acyl-L-carnitines could help to identify the patients before the phenotype conversion to AD and the patients who would benefit from the treatment with acetyl-L-carnitine. However, further validation on a larger number of samples in a longitudinal study is needed before application to clinical practice.
The aim of this study is to determine if there are differences in results of food allergen-specific IgE in patients from Zagreb (continental region) and Split (Adriatic region).

We evaluated food allergen-specific IgE in human serum in 500 patients from Zagreb and Split using AlleisaScreen® in vitro system. That is immunoblot assay for semi-quantitative determination of circulating allergen-specific IgE. IgE antibodies bind to the specific allergens, then these antibodies are bound to biotin and streptavidin conjugate leading to colour change. Concentration of specific IgE (the colouration of the allergen lines) is carried out by Improvio scanners and results are given in classes (from 0 to 6) and concentration (range 0 to 100 IU/mL).

Among 500 patients, one food allergen reported 11% (54/500) patients in Zagreb and 10% (50/500) in Split. More than one food allergen in Zagreb reported 29% (143/500) and 6% (30/500) patients in Split. Most common group of allergens in Zagreb and Split are vegetables. Most common single food allergen in Zagreb is F218 Bell Pepper and in Split Horseradish Peroxidase (CCD 2).

Comparison of the results demonstrates slight differences but large number of food allergies represents a significant health issue. Labeling of allergenic food is an important public health problem and the presence of “hidden” or undeclared allergenic ingredients are a great health risk. Because of that it is necessary to introduce a reliable method for detection and quantification of food specific allergens that can be applied in a fast and easy-to-use manner in daily practice.
Aim: Factor Xa-inhibitors (FXaI) apixaban (Apix) and rivaroxaban (Riva) are used in thrombophylaxis after orthopedic surgery. Few studies use patient samples measuring coagulation. Methods: Study group consisted of 20 unilateral total hip arthroplasty (THA) patients with prophylactic Riva (10 mg od) and 22 with Apix (2.5 mg bid). We collected blood samples before and 3 h after drug intake at 4 time points: preop, postop day 1, week 1 (day 2-8) and day 28. APTT, PT, albumin, Hgb, CRP and leukocytes were analyzed. Calibrated anti-FXa activity, and thrombin generation (TG, Calibrated Automated Thrombogram) assays were subsequently performed. Results: Hgb and albumin reached minimum at week 1, 105 g/L (range 78-132 g/L) and 29 g/L (range 24-37 g/L), respectively, while CRP and leukocytes increased. APTT and PT correlated poorly with Riva (APTT R2=0.07, PT R2=0.44) and APTT with Apix (R2=0.07), remaining mainly within the reference interval. Mean Apix concentration varied 8-fold, 19-153 ng/mL at peak, whereas Riva only 1.5-fold, 111-183 ng/mL. Both FXals prolonged TG lag time at all time points (p<0.001). Riva decreased ETP at all time points, reaching minimum at day 28 (536 nM/min at Riva 184 ng/mL, p<0.001). Yet, with Apix, ETP did not change until at day 28 (990 nM/min at Apix 112 ng/mL, p=0.01). Conclusions: PT and APTT lacked sensitivity to FXals. Riva attenuated TG outright, but ETP did not decrease with Apix until day 28. The inflammatory, thrombogenic state of the THA patients influences TG, contributing to the differences between FXals.

Abstract number 0106

Vanadium compounds modify function of blood platelets in diabetic patients

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Hyperglycemia may cause blood platelets hyperactivity and be a risk factor of thrombosis observed in diabetic patients. Vanadium compounds exert insulin-tropic properties without the effect of hypoglycemia, however vanadium influence on blood platelets function and metabolism has not been defined yet. The aim of this study was to investigate effects of organic bis(maltolato)oxovanadium(IV) (BMOV) and inorganic vanadium (III) chloride (VCl3) on platelets function and metabolism in healthy and diabetic patients. Blood platelets were isolated from citrated blood collected from 10 healthy volunteers and 10 diabetic patients with type 1 and 2 diabetes mellitus, then incubated in the presence and absence of BMOV (0.5mM) and VCl3 (0.5mM). Platelet aggregation (turbidimetry, spectrophotometry) was assessed. Thrombin-evoked aggregation (0.1IU/mL) was 72.1±3.6% and 63.2±3.1% in healthy and diabetic patients, respectively. BMOV and VCl3 had no effect on platelets aggregation in healthy subjects while aggregation was reduced by 15% (BMOV) and increased by 7% (VCl3) in diabetic patients. Collagen-induced aggregation (5/mL) was comparable in healthy and diabetic subjects. Moreover, organic and inorganic vanadium compounds effects are stronger in diabetic patients. These data demonstrate that BMOV inhibits platelets function by reducing thrombin- and collagen-evoked aggregation, while VCl3 increases platelet function in healthy and diabetic subjects. Moreover, organic and inorganic vanadium compounds effects are stronger in diabetic patients. These study shed a new light on safety of vanadium supplements used in diabetic patients. Supported by ST57 and MN16.

Abstract number 0182

Platelet morphology parameters – simple test of platelet reactivity assessment in patients on dual antiplatelet therapy

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Patient response to antiplatelet agents varies, and poor response may be associated with increased risk of ischaemic events. The aim of this study was to investigate the usefulness of platelet morphology parameters for monitoring platelet reactivity. Mean platelet volume (MPV) and large platelet count (L-PLT) have been studied together with platelet aggregation and platelet reactivity index (PRI) and P-selectin (CD62P) in patients with coronary heart disease (CHD) on dual antiplatelet therapy.

The study included 124 subjects with CHD and 25 healthy volunteers. Platelet aggregation was evaluated by light transmission aggregometry (LTA). PRI was determined by VASP(Vasodilator Stimulated Phosphoprotein) assay. L-PLT, MPV were performed using the method of two-dimensional optical platelets analysis. CD62P expression was measured by the immunocytosfluorometric method.

The number of patients “resistant” on aspirin-23% and clopidogrel-40% of total 124 subjects. CD62P expression showed a significant increase in total CHD group, CHD-r(resistant) and CHD-s(sensitive) subgroups vs. control (2689,68±169,89; 2707,56±153,68; 2648,12±362,82; 362,82±153,68; 362,82±153,68;
602.6±192.25 respectively). MPV and L-PLT were significantly higher in the CHD group vs. control group (7.7±0.3 vs. 8.9±0.2, 4.1±1.7 vs. 8.9±2.0 respectively). During the five-year observation of our 124 patients 11% demonstrated the presence of adverse cardiovascular events (+). In this group the mean value of MPV and L-PLT was higher compared with the group without the incident (-) (8.6±1.4 vs. 9.6±1.7; 8.5±5.1 vs. 12.7±4.0 respectively) both in LTA and VASP testing.

Measurement of MPV or L-PLT may be the standardized routine method beneficial in assessing the efficacy of clopidogrel/aspirin application in inhibiting blood platelets and thus enabling the prediction of clinical risk.

Abstract number 0224

Evaluation of pre-analytic and post-analytic phases of the coagulation and hemostasis laboratory in Hacettepe University hospitals

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Objectives: The pre-analytical and post-analytical phase in a test cycle contributes up to 75-90% of total laboratory errors. Rejection of unsuitable samples and critical value notification are the quality indicators of our laboratory according to Joint Commission International (JCI) accreditation standards. In this study, we aimed to evaluate the pre-analytical problems and critical values recorded on coagulation tests in 2015.

Materials and Methods: Blood samples for routine coagulation testing were considered unsuitable for analysis according to specimen rejection criteria of our laboratory. The coagulation tests were performed by BCS-XP system (Siemens Laboratory Diagnostics). The critical values in our laboratory were PT/INR >5, aPTT >100 seconds, fibrinogen <100 mg/dL, factor levels <5% and anti-thrombin III <50%.

Results: Total coagulation test request was 155,945 in one year and 5,090 tubes were rejected. The most common pre-analytical problems were referred as clotting (38.4%), following inappropriate volume (33.2%), inappropriate clinical orders (7.3%), misidentification (6.5%), hemolysis (6%), incorrect tube (3.8%), fibrin (3.3%) and delayed transport (1.5%), respectively. Among 155,945 tests performed in 2015, we reported 475 critical values. The critical value notification ratio was 56% for INR, 23% for factor levels, 12.2% for fibrinogen, 5.5% for antithrombin III and 3% for aPTT. Our critical value reporting rate was 97-99%, dropped call ratio was approximately 1-3% of all.

Conclusions: We detected an overall specimen rejection rate of 3.2% in coagulation laboratory. By documentation of rejected samples and periodic training of healthcare personnel, we expect to decrease sample rejection ratios below 1% and to improve quality management of the laboratory.

Abstract number 0226

Assessment of platelet functions in chronic myeloid leukemia patients treated with tyrosine kinase inhibitors

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Background: Bleeding is observed time to time in chronic myeloid leukemia (CML) patients using tyrosine kinase inhibitors (TKI). It has been suggested TKIs can disrupt platelet functions and cause bleeding without thrombocytopenia.

In this study, we aimed to evaluate platelet functions of CML patients using TKIs and examine in conjunction with bleeding symptoms to find out whether there is a correlation between them.

Methods: In this study, bleeding surveys were performed for 68 CML patients in chronic phase receiving imatinib (n=47), dasatinib (n=15) and nilotinib (n=6). Hematology analyzer LH780 (Beckman Coulter) was used for measurement of WBC and hemoglobin. BCS XP coagulation analyzer (Siemens) was used for measurement of PT, aPTT, TT, fibrinogen and factor assays. Light transmittance aggregometry (Chrono-Log) was used for the evaluation of platelet functions.

Results: The median age was 67 years (range, 18-78 years) in CML patients. WBC and hemoglobin levels, coagulation test results including PT, INR, aPTT, TT, fibrinogen and Factor VIII were in normal range. We observed that 25.6% of the patients using imatinib and 20% of the patients using dasatinib have minor bleeding symptoms. In Nilotinib treatment group, no bleeding symptom was observed. In platelet function tests, aggregation was induced by ADP, epinephrine, collagen and ristosetin, and secretion type defect was observed in 26% of the patients. No correlation was observed between platelet function defects and bleeding symptoms in these patients.

Conclusion: Platelet aggregation disorders can be observed in CML patients treated with TKIs. However, platelet dysfunction is not associated with bleeding disorder.
Inhibitory influence of pro- and active form of MMP-2 on platelet aggregation and adhesion to fibrinogen

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Research studies carried out on washed platelets suggested that the MMP-2 may contribute to the thrombotic complications of atherosclerotic plaque rupture. The aim of our study was to establish the influence of MMP-2 on adhesion and aggregation of platelets suspended in autologous plasma.

Methods: Platelet function was assessed in PRP (Platelet Rich Plasma). The influence of MMP-2 (in concentrations simulating local increase) on platelet function was examined using light transmission aggregometry and the microtiter immunoassay assessing the static adhesion to fibrinogen. In adhesion studies, the influence of MMP-2 on adherence to fibrinogen of activated or non-activated platelets was assessed immunologically. In aggregation studies, thrombin, ADP, collagen, and arachidonic acid (AA) were used as agonists.

Results: The pro- and active form of MMP-2 had a dose-dependent inhibitory effect on the thrombin and AA-induced aggregation when high concentration of enzyme was used (up to 100% of inhibition). When ADP or collagen were used as agonists, neither proMMP-2 nor aMMP2 had significant influence on platelet aggregation. Regardless of whether the activated or non-activated platelets were used, the aMMP-2 had a dose-dependent inhibitory effect (mean 54.8%) on platelet adhesion to fibrinogen. The proMMP-2 had no effect on thrombin-activated platelets adhesion to fibrinogen, but interestingly, the highest used concentration of the enzyme (1000 ng/ml) enhanced (mean 23.5%), while the lowest used concentration (0.1 ng/ml) inhibited (mean 26.5%), an adhesion to fibrinogen of the non-activated platelets.

Conclusion: MMP-2 in concentrations simulating local increase, prevent the adhesion to fibrinogen and AA and thrombin-induced aggregation of platelets.

Comparison of methods

Six sigma evaluation of D-100 and G8 HbA1c analyzers

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Background: Six Sigma (σ) Model is a global quality management system applicable to any production process. In clinical laboratories its application reports process efficiency by determining its variability, which influence not only productivity but also other relevant factors such as clinical impact on patient assessment. Glycosylated hemoglobin (HbA1c) is an ideal candidate for the application of the Six σ model. It is a longitudinal parameter in which small deviations cause a major clinical impact.

The aim of the study: Use the Six σ model to evaluate the HbA1c analyzers G8® and D-100®.

Material and Methods: Two HbA1c analyzers were evaluated: G8® from Tosoh and D-100® from Bio-Rad. For 20 consecutive days we proceeded to measure the three levels of Liquicheck® Diabetes Control, an external quality control material, in both instruments. We calculated the imprecision (CV%) and bias and estimated σ values considering different quality specifications (Total Admissible Errors, TEa).

Results: D-100: Bias = 2.33%; CV = 0.64%. G8: Bias = 1.63%; CV = 1.33%. D-100 σ values (TEa%, σ): (2%, -0.52); (3%, 1.05); (4%, 2.62); (6%, 5.76); (7%, 7.32). G8 σ values (TEa%, σ): (2%, 0.28); (3%, 1.03); (4%, 1.78); (6%, 3.29); (7%, 4.04).

Conclusion: D-100 has a better performance, reaching World Class category (σ ≥6) for TEa = 7%. For the same requirement G8 only reaches a good performance (σ ≥4). Considering the higher quality requirements (TEa < 3%, SEQC), σ values for both instruments are around 1, which is an unacceptable performance.
Abstract number 0094

Comparison of glycated hemoglobin (HbA1c) measurements performed on high-performance liquid chromatography (HPLC) and capillary electrophoresis (EC) autoanalyzers

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Aim of the study: According to NGSP and IFCC standardization programs high-performance liquid chromatography (HPLC) and capillary electrophoresis (EC) are certified methods of glycated hemoglobin (HbA1c) assay. The aim of study was to compare HbA1c measurement obtained on commercially available HPLC and EC autoanalyzers.

Materials and methods: Study included 110 whole blood samples collected from diabetic patients (n=62) and healthy volunteers (n=48).

Fasting venous blood was drawn using sterile plastic tubes with potassium-EDTA and stored at 2-8°C for no longer than 24 hours. HbA1c was assayed by HPLC method on Bio-Rad D-10 analyzer (Bio-Rad Laboratories, CA, USA) and by EC method on MiniCap Flex Piercing analyzer (Sebia, Lisses, France).

Results: In whole group median HbA1c concentration measured by HPLC and EC method was 47.5 mmol/mol (33.3-58.5) and 44.3 mmol/mol (32.2-56.3), respectively (p<0.001). In diabetic and healthy subjects HbA1c concentration assayed by HPLC method was significantly higher than measured by EC method (p<0.001 and p=0.004, respectively). Repeatable results were obtained in 16.4% of subjects, while 71.8% of results measured by EC method were decreased when compared with HPLC method. Although measurements were characterized by high concordance with correlation coefficient of 0.982 (95% CI: 0.975-0.987) and bias correction factor (accuracy) of 0.991, the Bland-Altman plot analysis showed a negative bias of -4.1% (95% CI: -13.8-5.6%) between results obtained by EC and HPLC methods.

Conclusions: HbA1c measurements performed by EC method on MiniCap Flex Piercing analyzer generate significantly decreased results when compared to HPLC method on D10 analyzer.

Abstract number 0189

Evaluation of four hemoglobin A1c instruments

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Hemoglobin A1c (HbA1c) is widely used for diagnosing and monitoring of patients with diabetes mellitus. Hence, HbA1c quantification methods must be robust, reliable and efficient. The aim of this study was to evaluate four analyzers: HA-8180 (Menarini), D-100 (Bio-Rad), G8 (Tosoh) and Capillarys2 flex piercing (Sebia).

Accuracy was evaluated on two levels of EQA material. Within-run and between-run imprecision (n=10) were assessed by using two patient samples and linearity by using two samples mixed in different proportions to expected HbA1c values ranging from 31-77 mmol/mol. Method comparison was performed on 128 patient samples (including 5 patients with hemoglobin variants). Each instrument was also individually compared with the in-house HA-8160 (Menarini). Usability and ergonomics of the four analyzers were evaluated with a scoring system.

Analytical precision was excellent and comparable on the four analyzers. Using the IFCC units (mmol/mol), within-run and between-run imprecision ranged from 0.56-1.11% for HA-8180, 0.98-3.09% for D-100, 0.47-1.78% for G8 and 1.70-2.86% for Capillarys2. The accuracy was good for HA-8180 and Capillarys2 (bias <2.3%), however for G8 and D-100 a greater bias was found, 3.7984.13% and 2.35876.8% respectively. All analyzers showed an acceptable linearity (r²≥0.95). Method comparison showed the HA-8160 is unable to measure HbA1c correctly in samples of patients with hemoglobin variants. All four analyzer correlated well with HA-8160 and with each other (r≥0.98). The D-100, followed by HA-8180, is the analyzer with the highest score on usability.

To conclude, the four analyzers met the analytical performance specifications. The D-100 and HA-8180 were superior in usability.

Abstract number 0232

To block or not to block, that is the question

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Immunoassays are vulnerable to analytical interference from heterophilic antibodies. Treatment with heterophilic blocking tubes (HBT) is one of several possible approaches to investigate for potential interference. An altered result after treatment with HBT is considered to be caused by heterophilic antibodies. We assumed that the assay format plays a pivotal role for the interpretation of a HBT-result.
We selected randomly 23 routinely drawn serum samples. Testing for thyrotropin and testosterone as an example for a "sandwich" assay format and a competitive assay format, respectively, was done. For both assay principles two assays of different composition were used. Sera were analyzed before and after treatment with heterophilic blocking tubes (Scantibodies). Results were reported as percentage recovery in the treated compared with the untreated serum.

The median (CV) values after HBT treatment for testosterone were 102 (8)% with the Testosterone II assay on cobas e411 (Roche) and 948 (142)% with the Access Testosterone on DxI800 (Beckman Coulter). For thyrotropin 98 (15)% for the Access TSH (3rd IS) and 83 (29)% for the Access HYPERsensitive hTSH both on DxI800.

Both assays with goat anti-mouse IgG coated paramagnetic particles exhibited significant deviation from the expected 100% recovery. Since the prevalence of heterophilic interference in routine assays is around 0.05%, it is highly likely that the difference in the results occurred mainly due to the assay components. For the competitive assay format this effect was obvious, but we presume that it occurs also in the "sandwich" assay and recommend to question the usability of HBT.

Critical values

Abstract number 0244

Does repeat testing of critical values improve their accuracy?

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Aim: Critical values are those results that indicate a high risk of morbidity or mortality in the absence of rapid intervention to treat the abnormal value or its underlying cause. The aim of our study is to determine the effect of repeat testing of critical values improve their accuracy.

Methods: 424 repeated critical laboratory values in 8 different hematology and chemistry tests including; hemoglobin, white blood cell (WBC), platelet, international normalized ratio (INR), glucose, potassium, sodium, calcium were examined, retrospectively. The absolute difference (first critical value – repeated value) and the percentage of change between the two tests [(absolute difference /first critical value)*100] for each critical value were calculated and then compared with the College of American Pathologists/Clinical Laboratory Improvement Amendments (CAP/CLIA) allowable error. All statistical analysis was done by SPSS version 16.

Results: The lowest percentage of change between the first and repeat tests was 1.1% for WBC >10000/mm3 and the highest was 8.9% for platelets ≤ 40000 / mm3. All percentage of changes between the first and repeat tests were under the CAP/CLIA allowable errors (hemoglobin 1.2%, WBC 1.1%, platelet 6.8%, INR 2.3%, glucose 2.9%, potassium 2.2%, sodium 1.3%, calcium 1.4%, respectively).

Conclusion: Repeat testing of critical hemoglobin, WBC, platelet, INR, glucose, potassium, sodium, calcium results have no benefit to increase their accuracy.

Education

Abstract number 0304

Laboratory medicine in pregradual study of general medicine

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Lack of knowledge and skills in laboratory diagnostics identified during the courses of clinical disciplines in 5th and 6th year of study of general medicine at Slovak Medical University was the main driver for the introduction of the new course „Laboratory Medicine“ (LM). Curriculum: A Principles of Laboratory Diagnostics (clinical laboratory, reference intervals, diagnostic value), B Laboratory Tests (in: molecular pathology, clinical chemistry, medical microscopy & urine analysis, cytogenetics, HLA, hematology, coagulation, immunopathology, transfusion medicine), C Rational Use of Laboratory Tests (in: internal medicine, endocrinology, hematology, coagulation, transfusiology & blood bank, extravasates, immunology, infection diseases, urgent medicine). Goals: acquiring the knowledge and skills in the field of A Clinical Laboratory (importance and use), B Laboratory Tests (pathophysiology, clinical and laboratory context), C Rational Use of Laboratory Tests (overuse, underuse misuse). LM is studied in the 7th semester (lectures: 14 hours, seminars 14 hours): it is credited by 2 credits.

Abstract number 0305

Rational use of laboratory tests: the Slovak experience

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During 1983-2015 4 initiatives of endeavour to remedy the situation in rational use of laboratory tests were undertaken. Hospital Pilot Study (1983) in duration of 2 years based on daily clinical ward visiting and providing education and feedback as well as subsequent monthly evaluation of outcomes has monitored the rational use of laboratory tests. In Consensus Study of Biochemical Diagnostics in Internal medicine (1998) twins of authors (laboratorian/clinician) for each field (cardiology, pulmology etc) were defined and worked out 3 categories of biochemical tests at national level. In Guidelines of Standard Diagnostic Procedures for Laboratory Diagnostics (1998) the proposal of proper laboratory diagnostic process in hematology, clinical chemistry, microbiology, immunology and radiology was presented. Monography of Rational Use of Laboratory Tests (2015) defines diseases states in which ordering of the given laboratory test is authorised in internal medicine, endocrinology, infections, and molecular diagnostics. In „Hospital Pilot Study“ (1983) 15 % decrease was achieved. In „Consensus study“ (1998) „what must, should, could be tested“ was defined, but paradoxically: no consensus was achieved. In „Guidelines“ (1998) though issuing the mononography zero clinical implementation was achieved. According „Rational UseMonography“ (2015) in a short pilot retrospective study from 43 680 evaluated tests according to defined criteria 40 % (17 315) were regarded as not justified.

Hemoglobinopathies

Abstract number 0127

Analysis of hemoglobinopathies from glycated haemoglobin chromatogram revision

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Hemoglobinopathies are a group of inherited autosomal recessive hemoglobin disorders. Their clinical repercussion goes from asymptomatic to severe status like drepanocitic anemia. The aim of this study is the analysis of the hemoglobinopathies in our area from glycated hemoglobin (HbA1c) chromatogram revision and hematological parameters.

Methods: All the samples with HbA1c received during 1 year (May 2015- April 2016) were chosen. Samples which had HbA1c determination in the last 3 months (2519) were not analysed. 80925 samples were finally analysed using HPLC method on the HA-8180 (Menarini Diagnostics). Those who had variant hemoglobin were then analysed on the HA-8160 (Menarini Diagnostics), whose analysis time is 4.2 minutes (in comparison with 1.5 of the HA-8180) and allows the separation and simultaneous quantification of different types of hemoglobinopathies.

Results: 32 patients had a significant hemoglobinopathy. They were classified as: 4 beta-thalassemia (12.5%), 13 Hb C (40.6%), 8 Hb S (25 %) and 7 Hb F (21.9%). Furthermore, 19 (59.4%) were autochthonous and the other 13 (40.6%) were immigrant population. The hemoglobin the immigrants had was HbA2 (8%), Hb C (46%) and Hb S (46%).

Conclusion: For some time now, our area has been a receptor zone of migratory population from Africa and Middle East. The quantification of glycated hemoglobin by HPLC is being determinant in the diagnosis of hemoglobinopathies which can be unnoticed by the clinician. Although the majority have heterozygous state, control program is important for prevention and treatment of these increasing pathologies.

Inflammatory diseases

Abstract number 0058

Decreased serum eicosapentaenoic acid levels and elevated secretory phospholipase A2 activity in acne vulgaris patients reveals a proinflammatory state

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Background: Acne vulgaris is an inflammatory skin disease of unknown etiology. The aim of this study was to determine circulating levels of polyunsaturated fatty acids (PUFAs) and assess serum activity of sPLA2 in patients with acne vulgaris.

Methods: Serum from 21 control subjects and 31 acne vulgaris patients with moderate and severe disease was evaluated for levels of arachidonic acid (AA, C20:4n-6), dihomo-gamma-linolenic acid (DGLA, C20:3n-6), eicosapentaenoic acid (EPA, C20:5n-3) and docosahexaenoic acid (DHA, C22:6n-3). Disease activity was determined according to the National Health Service guidelines for the management of acne. Lipid profile, routine biochemical and hormone parameters were assayed using autoanalyzers. Serum PUFA levels were measured by an optimized multiple reaction monitoring (MRM) method using ultra fast-liquid chromatography (UFLC) coupled with tandem mass spectrometry (MS/MS).

Results: No significant differences were found for the lipid profile, routine biochemical and hormone parameters between acne vulgaris patients and controls. Serum EPA levels were significantly decreased while activity of sPLA2, AA/EPA and DGLA/EPA ratio were significantly increased in acne vulgaris patients compared to age and gender matched controls. Serum levels of AA, DGLA and DHA showed no significant difference between acne vulgaris patients and controls.

Conclusion: The results of this study reveal the presence of a proinflammatory state in acne vulgaris as shown by significantly decreased serum EPA levels and increased activity of sPLA2, AA/EPA and DGLA/EPA ratio.

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Abstract number 0059
Vitamin D deficiency prevalence in inflammatory disease

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Background: In recent years, immune and inflammatory benefits of Vitamin D have been studied. The aim of this study is to associate inflammatory status with vitamin D deficiency.

Methods: Cross-sectional study. Vitamin D deficiency was established in 20 ng/mL according to American Endocrine Society definition. C-Reactive Protein levels less than 5 mg/dL, between 5 and 30 mg/dL and more than 30 mg/dL were considered normal, mild and severe inflammatory status respectively. Trend analysis was performed with STATA13.

Results: A total of 2853 patients were studied from January 2010 to December 2014. Prevalence of vitamin D deficiency was 34.78% in summer, 41.70% in autumn (OR: 1.20 (95% CI: 1.10 to 1.31)), 52.36% in winter (OR: 1.51 (95% CI: 1.39 to 1.64)), 50.99% in spring (OR 1.47 (95% CI: 1.35 to 1.60)). Prevalence of vitamin D deficiency was increased by inflammation severity (40.75 % in non-inflammation, 50.00% in mild one (OR: 1.22 (95% CI: 1.16 to 1.29) and 67.62 in severe one (OR: 1.66 (95% CI: 1.51 to 1.82). This trend was observed in each season, although the prevalence of vitamin D was higher in summer than in winter between non-inflammatory status and severe one (OR 2.13 95% IC: 1.71 to 2.65) versus 1.45 (95% IC: 1.23 to 1.70) respectively.

Conclusions: Severe inflammation increases the prevalence of vitamin D deficiency. It is important to consider vitamin D levels in patients with this inflammatory status.

Abstract number 0262
Significance of determining fecal calprotectin in differential diagnosis of inflammatory bowel diseases

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Background: Fecal calprotectin (FC) is a marker of intestinal inflammation and its determination is very useful to distinguish organic from functional gastrointestinal diseases although the gold standard investigation to differentiate this diseases is endoscopy. The aim of our study was to confirm the difference between the concentrations of FC in patients with inflammatory and non-inflammatory bowel diseases.

Methods: FC was determined in samples of patients diagnosed with bowel diseases using enzyme immunoassay BÜHLMANN Quantum Blue® Calprotectin Extended.

Results: According to the manufacturer’s recommendations, the patients (n = 40) were divided into three groups: patients (n=18) with values below 50 μg/g (values not indicate inflammation in the intestine), patients (n=10) with values between 50 and 200 μg/g (can represent mild
organic disease) and patients (n=12) with values above 200 μg/g (values indicate active organic disease with inflammation in the intestine). Endoscopic data was collected from 17 patients. All the patients in the first group were diagnosed with non-inflammatory bowel disease and the most of the patients in the second had the same diagnosis (only one had Crohn’s disease). The diagnosis of most patients in the third group was Crohn’s disease.

Conclusion: Our preliminary results confirmed that determination of FC is a simple, non-invasive and reliable test which can distinguish non-inflammatory and inflammatory bowel diseases as it correlated well with the endoscopic results of the patients. Determining FC could spare medical cost as it could reduce the number of performed colonoscopies and the risk of later complications of the disease.

Toxicology

Abstract number 0308

Could agmatine act as an antidote in a single toxic dose chlorpromazine-treated rats?

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Aim of the study. This study was conducted to investigate effect of agmatine (AGM) as a potential antidote for chlorpromazine (CPZ) treated Wistar rats in a single toxic dose. Since CPZ in toxic dose exerts neurotoxicity reflected in irreversible damage and loss of neurons in the forebrain cortex, striatum and hippocampus, our study pointed to the potentially protective effect of AGM and its ability to reduce the toxicity of CPZ. AGM (4-aminobutyl guanidine) is the decarboxylation product of arginine and an intermediate in polyamine biosynthesis. It is well known that AGM is a putative neurotransmitter synthesized in the brain, stored in synaptic vesicles, accumulated by uptake, released by membrane depolarization, and inactivated by agmatinase.

Methods. Chlorpromazine was applied intraperitoneally (i.p.) in a single dose of 38.7 mg/kg body weight (BW). The second group of rats was treated with both CPZ (38.7 mg/kg BW) and AGM (75 mg/kg BW). Rats were sacrificed by decapitation 48 h after treatment. The concentration of CPZ was determined by high performance liquid chromatography-tandem mass spectrometry (HPLC MS/MS) in homogenized brain tissue. Results. In the CPZ+AGM group, CPZ concentration was significantly decreased in the forebrain cortex (p < 0.05) compared to the CPZ group of animals.

Conclusions. AGM interaction with CPZ leads to reduced distribution of CPZ in brain of rats. The data indicated that i.p. administered AGM exerted antidote action in toxic dose CPZ-treated animals.

Abstract number 0347

Decreasing of adverse effect of (R,S)-ibuprofen by enzymatic transformation

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Introduction: One of the most frequently used medicine within group of 2-arylpropionic acids (profens) in treatment of inflammation is (R,S)-ibuprofen. Enantiomers of this compound demonstrate different therapeutic activities. (S)-ibuprofen inhibits equally both cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2), while (R)-ibuprofen is much less potent than (S)-enantiomer in inhibiting the activity of COX-1, and even less towards COX-2. (R)-ibuprofen contributes to increased side effects affecting the gastrointestinal tract, normal lipid metabolism and the membrane function.

Aim of the study: The aim of the study was to obtain (S)-enantiomer of ibuprofen by enzymatic transformation with the application of lipases. Methods: Enantioselective esterification of (R,S)-ibuprofen with the use of lipases from Candida rugosa OF and MY was performed. Additionally, the influence of reaction time and effect of alcohol moiety were tested. Enzymatic reactions were monitored with the use of HPLC chiral stationary phases.

Results: High values of enantiomeric ratio (E in the range of 40.1–71.3) of the esterification of (R,S)-ibuprofen were obtained using lipase MY. The reaction performed with the application of lipase MY allowed to achieve (S)-enantiomer of ibuprofen with the high enantiomeric excess of product eep = 95%. Conversion of the reaction was c = 30.6% and enantioselectivity E = 58.9 after 126 h of incubation.

Conclusions: Less toxic for human health (S)-enantiomer of ibuprofen was obtained by enzymatic transformation.

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Laboratory management, accreditation and quality control

Abstract number 0055

Antibiotics: performance in the external quality control of the Clinical Pathology Department of Matosinhos Local Healthcare Unit (ULSM)

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Background: External Quality Control (EQC) is interlaboratorial. The result (Res) of each parameter performed at the participating laboratory is compared to the group’s consensus average.

Objective: Assess the performance of three antibiotics (AB), Vancomycin (V), Gentamicin (G) and Amikacin (A), determined at the Clinical Pathology Department of ULSM in the context of the EQC, Therapeutic Drugs Program (TDM) of RIQAS® by Randox®.

Materials and Methods: September 2015-February 2016; serum concentrations of V, G and A (TDM Program); Abbott Architect c 8000 systems®: Chemiluminescence - V and G/ Turbidimetry - A. Results are compared to set of laboratories with the same analyzer, methodology/reagent.

Results: V- In the 12 samples (Aa), minimum standard deviation index (SDI): 0.1; maximum SDI: +0.64; average SDI: 0.25/Minimum Target Score (TS): 80; Maximum TS: 115. G- In 12 Aa, minimum SDI:+0.01; maximum SDI: -0.49; average SDI: -0.26/ Minimum TS: 75; Maximum TS: 120 (6x); Average TS: 114. A- In 12 Aa, minimum SDI:+0.04; maximum SDI: -1.10; average SDI: -0.18/ Minimum TS: 72; Maximum TS: 120 (6x); Average TS: 105. TS acceptable if 50; SDI if <2.

Conclusion: Performance according to EQC of RIQAS® (Randox®) proved to be, at least, “Good” and “Excellent”. Stable methodologies. It is extremely important to monitor AB with tight therapeutic margins. Compared to previous cycle (March 2015-August 2015) an improvement in all Aa was identified. EQC enables to ensure reproducibility of results, check calibration of analytical system and to identify non-conformities that lead to corrective actions.

Abstract number 0091

Evaluation of long-term imprecision of automated complete blood cell count performed on Sysmex XN-9000 platform

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Background: Blood cell counters are an undeniable component of total laboratory automation (TLA) systems. Beside the sophisticated technology now available, which improves the throughput and decreases the turnaround time, the analytical performance continues to cover a central role in assuring the quality of results. Here we present data on long-term imprecision obtained on three Sysmex XN-9000 platforms for the complete blood cell count parameters (WBC, RBC, Hb, Hct, MCV, MCH, MCHC and PLT), working in parallel in our core-lab TLA.

Methods: By using the Bio-Rad Liquichek Hematology-16 Control Normal Level material, we collected daily data from April to December 2015. Monthly and cumulative CVs were calculated and compared with the performance specifications derived from biological variability (desirable and minimum level of quality).

Results: A total of 195, 194 and 205 measurements were performed on the three instruments, respectively, in a 9-month period. Overall, the three platforms performed with desirable levels of imprecision. MCH and MCHC represented exceptions, fulfilling with some difficulties the minimum quality level. Particularly, MCHC failed to reach this goal (i.e., a CV ≤0.8%) in 50% of the 26 evaluated working months. It is worth to mention that average values for all parameters obtained on the three platforms were equivalent, confirming their perfect interchangeability. Conclusions: Daily collection of internal quality control data performed for 9 months on three identical platforms shows that the XN-9000 analyser can guarantee a high throughput without affecting the analytical quality performance. An improvement in MCH and MCHC estimates is however desirable.

Abstract number 0100


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A national approach for a better laboratory test request in primary care in Spain

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Redconlab studies compare primary care laboratory tests demand between Health Departments (HD) in Spain, generating a final report, to show inter-departmental variability with the aim of improving appropriateness in tests request.

In every REDCONLAB study a call for data was posted via email. Each participating laboratory was invited to fill out an enrolment form, submit their production statistics and provide HD organizational data.

76 laboratories participated in year 2012, corresponding to 17679195 inhabitants. The final report suggested some recommendations to improve request in aspartate aminotransferase (AST), Immunoglobulin A (anti-gliadin antibodies (AGA), anti-thyroglobulin antibodies (TgAb), total bilirubin (tBil), γ-glutamyltransferase (GGT), iron, free thyroxine (FT4), eritrocite sedimentation rate (ESR) and urea tests. In the 2014 edition (110 laboratories, 27798262 inhabitants), every laboratory was asked to report what tests strategies had been implemented and the implementation date.

There were calculated both year differences in the following indicators results: he ratio of request of related tests. AST/alanine transaminase (AST/ALT); AGA/IgA anti-tissue transglutaminase antibodies (AGA/anti-TG); TgAb/anti-thyroid peroxidase antibodies (TgAb/TPOAb); tBil/ALT; γ-glutamyltransferase (GGT)/ALT; iron/ferritin; FT4/thyroid-stimulating hormone (FT4/TSH); ESR/C-reactive protein (ESR/CRP) and urea/creatinine.
Every indicator result improved in the strategy group; there was statistical difference in five: AST/ALT, tBil/ALT, iron/Ferritin, FT4/TSH, Urea/Creatinine.

A better request was reached in AST, tBil, iron, FT4, urea primary care request.

Our results show the usefulness of benchmarking studies as a base on which to support consequent strategies to improve primary care laboratory tests request and monitor through appropriateness indicators in big populations.

Abstract number 0112

Laboratory requesting patterns in emergency department in Spain: a two years study

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To compare two years Emergency Department (ED) requesting patterns in Spain, using appropriateness indicators, to try to investigate if behaviors have changed along years.

A call for data was posted on a website. Spanish laboratories willing to participate in the study were invited to fill out an enrollment form and submit their results online. We obtained production statistics for the year 2012 from emergency laboratories. Twenty chemistry tests ordered by ED physicians were examined in a cross-sectional study. Every patient seen in the ED of any of these institutions regardless of reason of consultation, sex and age was included in the study. Each participating laboratory was required to be able to obtain organizational data. The study was repeated in 2014. After collecting data, twenty appropriateness indicators were calculated: every test request per 1000 ED admissions. The differences in indicator results in both years were calculated by way of the U Mann-Whitney test analysis. A two-sided p ≤ 0.05 rule was utilized as the criterion for rejecting the null hypothesis.

In the 2012 study, 76 laboratories participated, corresponding to 6858546 ED admissions and 110 laboratories in 2014, corresponding to 10654075 ED admissions.

No significant differences were found in ED test request, except for brain natriuretic peptide (BNP) that increased its demand in year 2014. The differences in BNP request are very difficult to explain because different patients case mix in ED in Spain. There is a need to reduce BNP request variability through interdepartmental communication and cooperation.

Abstract number 0113

Big differences in primary care vitamin D test requesting in Spain

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The inter-practice variability in 25-dihydroxyvitamin (25(OH)D) requested by General Practitioners (GPs) and demand in two periods was studied, trying to find out differences, if (25(OH)D) was appropriately requested and potential savings.

Spanish laboratories at different departments across Spain belonging to the 17 autonomous communities (CCAA), were invited to report 25(OH)D requested by the GPs for the years 2012 and 2014. Number of 25(OH)D requested per 1000 inhabitants and the index of variability were calculated, and compared both edition results. In 2014 CCAA were grouped regarding 25(OH)D availability or non-availability for its request in primary care, and sunlight hours and compared 25(OH)D demand. It was calculated how many tests could have been not measured if the availability vitamin D group would have the same figures that the non-availability and the potential economical savings.

76 laboratories, participated in the 2012 edition and 110 in 2014 corresponding to 17679195 and 27798262 inhabitants (59.8% Spanish population). Demand was highly variable in both and significantly higher in 2014. The group with non-availability in 25(OH)D demand had fewer requests. Also when more sunlight hours. 173885 tests could have been not measured if the demand in the availability group would have approximated to the non-availability vitamin D group. The potential savings would have been 886813,5€.

A high variability and increased demand in 25(OH)D requested by GPs and a lower demand when more annual sunlight hours and no 25(OH)D primary care test request availability is reported, showing potential savings close to one million euros.

Abstract number 0116

Related indicators: potential savings if appropriateness indicators targets were achieved

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To compare two indicators results regarding ratio of request of related tests in primary care in a big population in Spain and the differences between autonomous communities (CCAA).

Spain is divided in 17 CCAA also divided in Health Departments (HD) that cover a geographic area and its population. Every HD has a laboratory that attends the needs of every inhabitant. Spanish laboratories were invited to fill out an enrollment form regarding numbers of requested tests, in primary care patients. Each participating was required to obtain patient data from local Laboratory Information Systems, number of aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatinine and urea requested in year 2014, and to provide organizational data.

Ratio of AST and ALT (AST/ALT), and urea and creatinine (UREA/CREA) demand were calculated, and the savings if AST/ALT and UREA/CREA would have reached indicators targets (0.25 and 0.1 respectively). Laboratories were grouped in the different CCAA, when more than 4 participants and indicators result were compared.

110 laboratories (27798262 inhabitants) participated. The savings if indicators would have reached the target, would have been 1168317€. 10 CCAA had more than four participants showing differences in both indicator results.

The differences in both indicators results in ten Spanish CCAA are difficult to be explained by different primary care patient’s case mix. Close more than million could have been saved if indicators targets would have been reached, suggesting the need to design strategies for a better request.
Abstract number 0131

Diagnostic accuracy of high fluorescence lymphocyte parameter in Sysmex XN-2000 hematology analyzers

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Background-aim: Along the blood-EDTA sample analysis some flags are generated to review. One of those flag generated on the Sysmex-XN analyzer is “Atypical Lympho?”, which detects reactive atypical lymphocytes by the percentage of high fluorescence lymphocytes (HFLC%). However, this flag produces many false positives, since not always their presence is confirmed in the smear. Aims:

1. Study the diagnostic accuracy of the HFLC% in relation of the reactive lymphocyte presence that show clinical significance
2. Select the best HFLC% cutoff to reduce the number of blood smear review.

Methods: 281 non-hematological whole-blood samples were analyzed by Sysmex XN-2000. Samples were selected according to “Atypical Lympho?” flag presence and checked by digital images obtained by CellaVision. True positives are confirmed when at least 10% of lymphocytes meet with the following morphological features: medium-large size, moderate nucleus-cytoplasm relation, rounded nucleus with little condensed chromatin and intensely basophilic cytoplasm. Finally, we calculated a ROC curve (MedCalc).

Results: The area under the curve (AUC) was 0.812 (p<0.0001), with a 95% confidence interval [CI]: 0.761–0.856. The best cutoff for HFLC% was 2% (sensitivity: S=76.92; Specificity: SPC=82.53).

Conclusions: Results suggest that HFLC% parameter is capable of distinguishing positive and negative cases of reactive lymphocytes. However, the degree of discrimination is not very high, so we decided to adjust the HFLC% cutoff at >1.5%, in order to decrease the number of false negatives (S=84.62; SPC=65.50).

Abstract number 0135

Intensive educational efforts towards primary health care increase patient identification practice in blood sample collection and thereby patient safety

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Aim: The aim of this study was to evaluate the effect of intensive educational efforts together with External quality assessment (EQA) of the pre-analytical phase from 2013 to 2015 in primary health care in Norway.

Methods: Pre-analytical EQA was sent yearly by Norwegian Quality Improvement of Primary Health Care Laboratories (Noklus) to General Practitioners’ (GP) offices and nursing homes (n=2000) from 2013 to 2015. In addition, pre-analytical issues and especially patient identification were a topic at every visit to all Noklus-participants by the Noklus laboratory advisors, at yearly courses and in newsletters sent to all participants twice a year. Furthermore, a monthly poster with a pre-analytical message was published at Noklus’ home page.

Results: The response rate varied between 42% and 55%. The percentages of participants asking for the patients’ name and the Norwegian identification number increased from 8% in 2013 to 35% in 2015. The increase was similar for those participating only once and those participating every year. The percentages of GP-offices asking for only the patients’ name decreased from 23% in 2013 to 14% in 2015 for those participating only in 2013 or the 2015 survey and for those participating in both surveys.

Conclusion: It seems that the educational effort more than the pre-analytical EQA influenced the actions and resulted in an increase in the percentages of participants that followed the guidelines for patient identification.

Abstract number 0139

Thyroid laboratory tests demand in Spain

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The aim was to study primary care thyroid laboratory tests demand and appropriateness at high National scale in Spain and the differences between autonomous communities (CCAA).

Spain divided in 17 CCAA is also divided in Health Departments that cover a geographic area and its population, with a laboratory that attends the needs of every inhabitant. Each participating laboratory was required to obtain patient data from local Laboratory Information Systems, number of thyroid tests in year 2014, and to provide organizational data. The request of every test per 1000 inhabitants and ratio of related test request free thyroxine (fT4/Thyrotropin(TSH), triiodothironine(fT3)/TSH, antitiroglobulin antibody (TgAb)/antiperoxidase antibody(TPOAb)) were calculated and compared in the different CCAA, with more than 4 participants.

110 laboratories, participated (27798262 Inhabitants). Close to 6 million of TSH have been requested, conforming an expense close to 10248340€. 10 CCAA had more than 4 participants. TSH request per 1000 inhabitants ranged from 118 to 289. fT4 more than doubled its result in the most demanded. TPOAb request per 1000 inhabitants ranged from 0.2 to 11.2, and TgAb and TPOAb request were redundant in 5 CCAA as shown by TgAb/TPOAb results.

There is a high request for thyroid laboratory tests in primary care in Spain. The request and expenses in TSH measurement are big. The variability observed between CCAA and inappropriateness, especially in antithyroid antibodies, suggests that are necessary the design of strategies at a National scale for a better request.

Abstract number 0141

Primary care request of anemia chemistry tests in in Spain

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The aim was to study primary care anemia laboratory tests demand at a National scale in Spain, the differences between autonomous communities (CCAA) and potential savings if more appropriate request.

Spain is divided in 17 CCAA also divided in Health Departments that cover a geographic area and its population, with a laboratory that attends the needs of every inhabitant. Each participating laboratory was required to obtain patient data from local Laboratory Information Systems, number of anemia chemistry tests in year 2014, and to provide organizational data. The request of every test per 1000 inhabitants and ratio of folate/B12 vitamin and transferrin/ferritin demand were calculated and compared in CCAA, with more than 4 participants. As ferritin is the primary care recommended marker for microcytic anemia, the saving if iron and transferrin approximate their requests for the CCAA with lower demand (reagent cost) was calculated.

110 laboratories, participated (27798262 Inhabitants). The request in ten CCAA was different for iron, B12 vitamin and folate. B12 vitamin and folate are requested together in the majority of CCAA. 71651€ and 1457429.7€ could have been saved in iron and transferrin measurement if every CCAA would have approximated its request for the one with less demand.

There is a high request of anemia chemistry tests in primary care in Spain and differences between CCAA. Despite the different conditions of B12 vitamin and folate to be demanded, they are requested together. The potential savings suggest that are necessary the design of strategies at a National scale for a better request.
Abstract number 0150

Demographic and laboratory pattern of primary care patients with anorexia

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Primary care is the first level the patient use to solve any medical problem. Anorexia as a symptom could be the first signal of an oncoming disease. The aim is to study the patient that attends primary care consultation because anorexia as a symptom, the requested laboratory tests and their results.

Seven year retrospective observational cross-sectional study: patients seen at a primary care center because anorexia, that had a laboratory request, the type and number of the ten most frequent requested tests and their pathological values was studied.

3562 patients attended primary care because anorexia as a symptom. To 20% a laboratory request was demanded. Patients with a laboratory request had a mean age of 33 years, 58% were women and 47% were younger than 16 year. Cell blood count, was demanded in every patient; glucose, creatinine, alanine aminotrasferase, and lipid metabolism tests in more than 90%; thyrotropin (TSH) in 80%. The most common pathological value was haemoglobin (18%). 6% of the patients presented ferritin low values. 20% when under 16. Abnormal TSH values were observed in 2% that increased when below 16 years (9%).

One of every 5 patients with anorexia had a laboratory request. The frequency of abnormal results in ferritin and TSH in young population and its adverse effects regarding poor development outcome and performance in cognitive functions show the convenience of ordering a blood test to all patients under 16 attending primary care because this symptom.

Abstract number 0152

Most requested primary care tests laboratory requests in Spain

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The aim was to study the demand from primary care of the most requested laboratory test in a big population in Spain and the differences between autonomous communities (CCAA).

Every Spanish CCAA is divided into Health Departments (HD) that cover a geographic area and its population. A laboratory attends the needs of every HD inhabitant. Spanish laboratories were invited to fill out an enrollment form and submit their results online. Each participating was required to be able to obtain patient data from local Laboratory Information Management System Patient’s database. The primary care 2014 request of the 10 standard most requested laboratory tests in the previous Redconlab edition was reported: alanine aminotransferase, aspartate aminotransferase, total cholesterol, creatinine, gamma glutamyl transpeptidase (GGT), glucose, HDL-cholesterol, triglycerides, uric acid and urinalysis. Test-utilization rates were calculated as tests per 1000 inhabitants. Laboratories were grouped in the different CCAA when more than 4 participants and results compared.

110 laboratories participated, corresponding to 27798262 inhabitants (59.8% Spanish population) from 15 CCAA and 82710869 demand of the 10 standard most requested tests; 13822640 if extrapolating the entire Spanish population. Among the ten evaluated tests AST, GGT and uric acid showed the greatest variation in its request between 10 CCAA.

The demand from primary care of the most requested laboratory test in a big Spanish population was very high showing differences in the request between CCAA that make necessary the design and establishment of strategies at a National scale through the interdepartmental cooperation and communication.
Abstract number 0154

Great differences in the request of glycated hemoglobin in primary care in Spain

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The study was performed to compare glycated hemoglobin (HbA1c) request from Primary Care at different autonomous communities (CCAA) in Spain.

Spain is divided in 17 CCAA also divided in Health Departments (HD) that cover a geographic area and its population, with a laboratory that attends the needs of every inhabitant. 110 Spanish laboratories from diverse regions across Spain filled out the number of HbA1c tests requested by general practitioners (GPs) during the year 2014. Every patient seen at the different primary care centers was included in the study. Each participating laboratory was required to provide the HbA1c request and HD organizational data. The number of HbA1c requests per 1000 inhabitants was calculated and compared in the different CCAA, with more than 4 participants.

2779826 patients were included in the study, corresponding to 60% of the Spanish population. A total of 1 2651618 HbA1c tests were ordered. Significant differences were noticed between ten Spanish CCAA (p<0.001). HbA1c demand doubled from the least to the most demander CCAA.

There was a high variability regarding the Primary Care request of HbA1c. The differences in the indicator results at ten Spanish CCAA are difficult to be explained by different primary care patient’s case mix. This emphasizes the need to accomplish interventions to improve an appropriate use.

Abstract number 0317

Does “not re-requesting a rejected test” show test overutilization?

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As the variety of lab tests, speed of time to reach the results and the quality of test results have increased, it also brought along a new problem, test overutilization. We analyzed our hospital’s LIS to see the rate of re-requesting after sample rejection by the lab. Last 10 months rejection statistics were examined. 24 hour time period is taken as a cut-off for not requesting the test again. The clinical biochemistry, immunoasay, hematology, coagulation and cardiac tests of only the emergency room are included in the study. The first three tests that were not re-requested after rejection and their rate of percentages were ammonia (64%), fibrinogen (62%) and aPTT (46%). The first three tests that were re-requested after rejection and their rate of percentages were electrolytes (Na with K) (83.7%), creatinine with BUN (83.3%) and glucose (82.8%). The mean percentages of not requesting after rejection for troponin and CK-MB Mass were 24% and 42%, respectively. As a result when samples are rejected due to preanalytical errors, same tests should be re-requested by the clinics if they were really necessary. We think that these high percentages of not re-requesting the rejected tests show us the unnecessary test requesting which is an important expense item covering all hospitals.
Laboratory testing in Emergency Department

Abstract number 0084

Amylase and lipase in the diagnosis of acute pancreatitis in the emergency department

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Stat laboratories are under pressure to supply information as quickly as possible for the diagnosis and treatment of disease. No single laboratory test or clinical sign is pathognomonic for acute pancreatitis; there are many bio-markers and inflammatory mediators. The aim is through applying the plan–do–check–act cycle, to study the better laboratory markers algorithms for pancreatitis diagnosis as a tool to enhance the contribution of the clinical laboratory in the emergency department (ED).

The plan–do–check–act cycle was applied consecutively in two occasions. In the first cycle lipase and amylase were measured individually. Lipase was used in the second cycle as a first line test and the amylase was added when abnormal results. The economic study was conducted by calculating the cost of laboratory (reagent used for every amylase and lipase measurement) of each case of diagnosed pancreatitis.

When analyzed individually, the sensitivity and specificity of amylase were 0.70 and 0.96; 0.85 and 0.96 for lipase. The area under the curve was 0.870 for amylase and 0.963 for lipase. In the second cycle, sensitivity and specificity were 0.92 and 0.98. Each case of pancreatitis showed a laboratory cost of 45.2 euros. By only using amylase, the theoretical cost would have been 63.7.

Through consensus and the use of quality tools is possible to improve the laboratory contribution to the diagnosis of acute pancreatitis in patients attending ED.

Laboratory testing in pregnancy

Abstract number 0130

Impact of a protocol change on the miscarriage detection from the laboratory

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The screening of the first term in pregnant women is a practice done in order to detect aneuploidies. Recently, our laboratory protocol has changed. The aim of this study is to associate the protocol change with the increased prevalence of miscarriages detected by the laboratory.

Methods: Data from January to March 2015 and from January to March 2016 was collected from the laboratory database. Laboratory reports of miscarriages were registered and confirmed by checking the patient’s medical record. In 2015 the gynaecological protocol included simultaneous echography and biochemistry analysis between the 10th and 13th weeks. The new protocol leads to perform the biochemistry test in the earlier weeks (8-10) and the echography in the latest ones, in order to take advantage of the most sensitive period of each test.

Results: 2087 pregnancies have been studied. Miscarriage report prevalence was 0.94% in 2015 and 5.04% in 2016. Prevalence rate was 5.34 (95% CI: 2.66 a 10.74) with significant differences (p<0.001). 53 cases would not have been detected with the previous protocol (IC95%: 35.6 a 48.2 cases).

Conclusion: The increased number of spontaneous miscarriage reports during 2016 is associated to the protocol change due to the biochemistry test advance in timeline. With the previous protocol less number of miscarriage were reported.

Metabolic diseases

Abstract number 0070

MMP-10 and MMP-1 in serum and saliva of patients with different body-mass index

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Background: Obesity is a multifactorial chronic disease and its incidence has been increasing dramatically. Their development is associated with extensive modifications in adipose tissue involving adipogenesis, angiogenesis, and extracellular matrix (ECM) remodeling. The Matrix Metalloproteinases (MMPs) are proteolytic enzymes that are able to cleave ECM components and non-ECM proteins. MMP-10 is activated by serine proteases and its active form is able to activate other MMPs (MMP-1 and MMP-8). MMP-10 has been studied in other inflammatory diseases, however, studies of MMP-10 in obesity don’t exist. Therefore, it becomes important to understand the consequences and damage of obesity, through the evaluation of biomarkers that can reflect the individual inflammatory state.

Aims: Assess the levels of MMP-10 and MMP-1 in the serum and saliva, and correlate both levels in individuals with different body-mass index.

Methods: The study included 72 individuals classified by body fat percentage and divided in 3 groups: normal weight, overweight and obese. The semi-quantification of MMP-10 and MMP-1 in serum and saliva was performed by slot blot technique.

Results: The individuals with the highest percentage of body fat showed higher levels of MMP-10 in saliva and lower levels in serum. The normal weight individuals presented higher MMP-1 levels in both fluids. Were observed positive and statistically significant correlations between the levels of MMP-10 and MMP-1 in both fluids.

Conclusions: MMP-10 and MMP-1 are altered in obesity and appear to be implicated in ECM remodeling. Saliva showed to be a fluid with potential for disease monitoring.

Abstract number 0093

A cross-talk between nitric oxide and forskolin in the regulation of blood platelet function in diabetes

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Chronic hyperglycemia in diabetes leads to impaired platelets activity and plays a role in the pathology of diabetic complications. Platelet activation is down-regulated by nitric oxide (NO) and prostacyclin in a cGMP and cAMP-dependent signaling pathways, respectively. The aim of the study was to investigate the effect of activators of those pathways on platelet function in healthy subjects and diabetic patients and to verify the hypothesis whether the parallel activation of both pathways can lead to a cumulative down-regulation of platelet function in hyperglycemia. Blood platelets were isolated from citrated blood obtained from 11 healthy volunteers and 11 patients with type 1 and 2 diabetes. We tested the effect of compounds increasing intraplatelet cAMP (0.005M forskolin) and cGMP (0.01M SNP) on thrombin-induced (0.1IU/ml) aggregation by turbidimetry. The percentage of HbA1c was 60% higher in diabetic patients compared to healthy people (p<0.05). The thrombin-induced platelet aggregation was 64.0±6.51 in healthy volunteers and was similar to diabetic platelets. In healthy subjects, SNP inhibited aggregation by 20% (p<0.05), forskolin by 52%, while both compounds reduced the aggregation by 53% (p<0.001) compared to controls. In diabetic patients SNP inhibited aggregation by 29% (p<0.05), forskolin by 50% and the both compounds by 92% (p<0.001) compared to controls. SNP and forskolin had a similar effect on reduction of platelet aggregation in healthy and diabetic patients. However both compounds caused cumulative reduction of aggregation in diabetic platelets. Therefore, it seems that the data presented here may be important to plan an antiplatelet therapy in patients with a hyperglycemia. Supported by ST57 and MN116.

Abstract number 0175

Development of a clinical decision support system for diabetes care: a pilot study

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Management of complex chronic diseases such as diabetes requires the assimilation and interpretation of multiple laboratory test results. Traditional electronic health records tend to display laboratory results in a piecemeal and segregated fashion. This makes the assembly and interpretation of results related to diabetes care challenging.

We developed a diabetes-specific clinical decision support system (Diabetes Dashboard) interface for displaying glycemic, lipid and renal function results, in an integrated form with decision support capabilities, based on local clinical practice guidelines. The CDSS included a dashboard feature that graphically summarized all relevant laboratory results and displayed them in a color-coded system that allowed quick interpretation of the metabolic control of the patients. An alert module informs the user of tests that are due for repeat testing. An interactive graph module was also developed for better visual appreciation of the trends of the laboratory results of the patient.
In a pilot study involving case scenarios administered via an electronic questionnaire, the Diabetes Dashboard, compared to the existing laboratory reporting interface, significantly improved the identification of abnormal laboratory results, the long-term trend of the laboratory tests and tests due for repeat testing. However, the Diabetes Dashboard did not significantly improve the identification of patients requiring treatment adjustment or the amount of time spent on each case scenario.

In conclusion, we have developed and shown that the use of the Diabetes Dashboard incorporates several decision support features, can improve the management of diabetes.

Abstract number 0186

Vitamin D levels in black African adults at Aga Khan University Hospital, Nairobi

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Introduction: Vitamin D has been known for its benefits in bone health. Recent studies have demonstrated its benefits in non-communicable and communicable diseases.

The cut-offs for vitamin D are derived from a Caucasian population. Studies done among black African adults in Africa are few with deficiency ranging from 5-91%. Due to the differences in skin colour, latitude and vitamin D binding protein these values might be misleading.

Objectives: To determine the proportion of healthy black African adults classified as 25(OH)D deficient using a cut-off of 20ng/ml and to correlate this with markers of physiological deficiency.

Methods: A cross sectional study carried out among 258 blood donors from March to May 2015. Vitamin D levels were assayed and correlated with PTH, calcium and phosphate.

Results: The proportion of study participants were vitamin D deficient was 17.4% (95% C.I 12.73-22.07. The 25(OH)D level that coincided with an increase in PTH was 30ng/ml. There was no difference in calcium, inorganic phosphate between Vitamin D deficient and non-deficient individuals. Males were less likely to be vitamin D deficient (O.R 0.48 (95% C.I 0.233-0.933) p 0.04).

Conclusion and Recommendations: This study highlights the prevalence of vitamin D deficiency based on a widely adopted cut off. Suitable 25 (OH)D cut-offs need to be established in the African population to assist clinicians and researchers in interpreting vitamin D levels. Studies correlating 25(OH)D levels, 1,25(OH)D, vitamin D binding protein and physiological markers among Africans need to be done.

Abstract number 0195

The transition to menopause increases adiponectin concentration independent of BMI values

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Aim of the study: To evaluate the influence of menopausal status on the serum adiponectin concentration and investigate whether the adiponectin concentrations are related to BMI or FSH changes during menopausal transition in 5-years follow up study.

Methods: We investigated 80 women aged 40-60 years old who participated in 5-years follow up study. Women were divided into three groups: premenopausal women who reached perimenopause (PRE/PERI), perimenopausal women who reached menopause (PERI/POST), and women who were postmenopausal throughout the study (POST/POST). The anthropometric data, serum adiponectin (ELISA R&D) and FSH concentrations (AxSYM, Abbott Diagnostics) were used.

Results: The values of BMI, FSH and adiponectin were significantly higher in the group PRE/PERI [BMI 24.9 (3.0) vs 26.6 (2.7) p=0.05 and FSH 8.3 (7.3 -12.2) vs 12.4 (7.4 – 44.83) p=0.03 and adiponectin 9.5 (8,145,4) vs 11.4 (9,6 -18,4) p=0.035] and in the group PERI/POST [BMI 24.6 (3.1) vs 26.0 (3.3) p=0.005 and FSH 13.6 (9,119,0) vs 76.6 (50,7 – 119,7) p=0.004 and adiponectin 9.0 (6,8 - 15,6) vs 14.0 (9,6 – 20) p= 0.005] after 5-years of follow up. Adiponectin did not increase significantly in group POST/POST during the 5-years observation. In multiple linear regression analysis adiponectin changes showed the significant BMI-independent positive correlations with FSH change in group PRE/PERI (beta = 0.81 p<0.001) and in group PERI/POST (beta =0.6 p=0.005) after 5-years of follow up.

Conclusions: The transition to menopause increases serum adiponectin concentrations and this increase is more strongly related to the sex hormone concentration than to body mass change.
Abstract number 0238

MMP-7, -8 and TIMP-3 levels in serum and saliva of obese subjects

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Introduction: Obesity is a chronic disease, resulting in expansion of adipocytes and extracellular matrix remodelling, performed by matrix metalloproteinas (MMPs) and regulated by its tissue inhibitors (TIMPs). Some secondary injuries are caused by chronic inflammation related to obesity, through the break of the homeostasis between MMPs and TIMPs. Recently, it was shown that MMP-7 and MMP-8 may reflect anti-inflammatory or defensive potential in human inflammatory disease. There are few investigations about TIMP-3 in obesity and saliva is very poorly studied in obese young adults.

Aims: Determine the levels of MMP-7, MMP-8 and TIMP-3 in serum and saliva samples of normal weight, overweight and obese young adults.

Methods: The study included 72 subjects classified by body fat percentage. The semi-quantification of MMP-7, MMP-8 and TIMP-3 were performed by slot blot.

Results: The normal weight and overweight subjects showed lower levels of TIMP-3 in saliva samples comparing with the group of obese individuals. The ratio MMP-7 / TIMP-3, in saliva and serum samples, were higher in normal weight subjects comparing with the obese.

Conclusions: The higher levels of TIMP-3 in obese adults suggest that it plays an important role in the pathophysiology of obesity. The high ratio of MMP-7/TIMP-3 in the normal weight adults compared with the obese adults, in both biological fluids, suggest that MMP-7 play a protective role, being obesity associated with specific changes in the MMP/TIMP balance. The correlations between fluids allows us to propose saliva sample as an alternative to serum with great potential in early diagnosis.

Abstract number 0245

The importance of front-line tests in diagnosis of porphyrias: 12 years of experience in North Estonia Medical Centre

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Porphyrias is a group of rare metabolic disorders whose diagnosis depends on identification of specific patterns of porphyrins. It is usually necessary to have at least three tests for front-line diagnosis of porphyrias: urinary porphobilinogen, alfa-aminolevulinic acid and total porphyrins.

The aim of present study is to demonstrate the importance of front-line tests based on our experience.

Methods: Porphobilinogen, alfa-aminolevulinic acid and total porphyrins in urine were measured with ion-exchange columns (Bio-Rad). Data of diagnoses and analyses were recorded from HIS.

Results: Our laboratory receives samples for porphyrins measurements from all the hospital of the capital of Estonia Tallinn and also from the rest of the northern part of Estonia with approximately 2/3 of Estonian population in total. In 2004-2015 our laboratory performed 3651 analyses of porphyrins. The most samples from patients with suspicion of porphyras were sent to the laboratory due to gastroenterological, neurological and dermatological symptoms compatible with porphyria. The diagnosis of porphyria was established only for a small number (23) of all the numerous (1237) investigated patients, among them 13 patients were diagnosed with porphyria cutanea tarda and 10 with acute intermittent porphyria.

Conclusion: Because clinical presentation of porphyrias is often nonspecific, porphyria may be suspected in many patients who actually do not suffer from this disease, but who have symptoms compatible with porphyria. Our results emphasized once more the role of front-line biochemical tests in management of this severe disease: in most cases the right diagnosis were made without delay and patients received appropriate and timely treatment.
Abstract number 0247

Osteocalcin levels are related to glucose homeostasis

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Aim: Osteocalcin, as shown in animal studies, promotes insulin secretion and insulin sensitivity. We aimed to investigate circulating levels of osteocalcin in subjects with different glucose tolerance with respect to insulin sensitivity and adipokines.

Materials and Methods: Seventy-five subjects (58 women and 17 men; age range 30-65 years) were included. After an overnight fasting, a 75 g oral glucose tolerance test was performed and venous blood samples were drawn to determine osteocalcin (intact and uncarboxylated), glucose, insulin, adiponectin, and leptin levels. Except for glucose and insulin, analytes were measured by commercially available ELISA kits. Insulin sensitivity was estimated by Homeostasis model assessment (HOMA) model. Patients were grouped according to the results of their glucose tolerance tests as normal glucose tolerance (n=25), prediabetic (n=25) which included impaired fasting glucose and impaired glucose tolerance, and diabetic (n=25).

Results: Subjects were divided into tertiles according to their intact and uncarboxylated osteocalcin levels. Subjects in the upper tertile of uncarboxylated osteocalcin showed higher frequency of normal glucose tolerance and subjects in the upper tertile of uncarboxylated osteocalcin showed higher frequency of diabetes, but it did not reach a statistical significance. The upper intact osteocalcin tertile was associated with higher frequency of normal glucose tolerance. Prediabetic subjects displayed significantly lower carboxylated osteocalcin levels.

Conclusion: Our data suggest lower carboxylated osteocalcin concentrations are related to glucose homeostasis in humans.

Abstract number 0254

Available methods and techniques for the assessment of insulin resistance

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Insulin resistance is a state of inadequate muscle, fat and liver response to normal concentrations of insulin. This results in elevated blood fatty-acid concentrations, reduced muscle glucose uptake, and increased liver glucose production, and contributing to hyperglycaemia. High plasma glucose and insulin concentrations are features of metabolic syndrome and type 2 diabetes. Insulin resistance has been also linked with several other disease states like PCOS, vitamin D deficiency or hypercorticolism. HIV and tuberculosis are also associated with changes in insulin resistance. The ability to measure and quantify insulin resistance or insulin sensitivity is important for several reasons. It helps not only to understand the etiopathology of the metabolic syndrome, type 2 diabetes and other diseases associated with insulin resistance disease states but also to examine their epidemiology and assess the effects of intervention. There are number of well-established tests for the direct or indirect measurement of insulin resistance, which differ in complexity and labour intensity. Direct methods for measuring insulin sensitivity in vivo like hyperinsulinaemic euglycaemic clamp are complex, labour intensive and rely on steady-state analysis of glucose and insulin. Indirect methods are less complex and rely on frequently sampled intravenous tolerance test and minimal model analysis. The most commonly used methods due to their simplicity are surrogate indices for insulin sensitivity or resistance such as e.g HOMA, QUICKI. In this presentation, drawing on my practical experience, I will review the available techniques for the assessment of insulin resistance and insulin sensitivity and discuss their advantages, limitations and applications.

Abstract number 0280

Glycocerebrosidase enzyme activity in dried blood spot sample is closely related to the number of leucocytes

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Lysosomal storage diseases which are related to deficiency of specific lysosomal hydrolases resulted to clinical aspects due to accumulation of substrates in different tissues. Since Dried Blood Spot is non-invasive, low-cost and high enzyme stability compared to leucocyte or fibroblast culture, it’s recommended as a first screening test. However the false positive rate with DBS sample is higher compared to other samples. We aimed to investigate any possible effect of leucocyte number on enzyme activity in a retrospective study.
We re-evaluated the alpha glycosidase, glycerocerebrosidase, alpha galactosidase, sphingomyelinase, galactocerebrosidase and alpha-L-iduronidase enzyme activity results (n=220) in regard to leucocyte number among data within last 1 year. Enzyme activities have been measured by fluorometric and LC MSMS methods.

While glycerocerebrosidase and galactocerebrosidase positively correlated with the number of neutrophils, alpha galactosidase, sphingomyelinase and alpha-L-iduronidase positively correlated with the number of lymphocytes. Alpha glycosidase activity showed a correlation both lymphocytes and neutrophils. The patients having the glycerocerebrosidase enzyme activity which was lower than 0.6 nmol/mL/h (which is accepted as the cut off value to recall the patients) existed lower number of leukocyte, lymphocyte and neutrophil compared to those of patients having higher enzyme activity than 0.6.

Our data indicated that the enzyme activity in dried blood samples including low leucocyte number might be found lower than reference intervals resulting in false positive diagnosis. Therefore we suggest that the laboratory scientists should evaluate the number of leucocyte while they were interpreting data.

Abstract number 0323

The uncertainty of HOMA-IR in clinical practice

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The homeostatic model assessment - index of insulin resistance (HOMA-IR) is predictive risk factor for cardiovascular disease and metabolic syndrome. The uncertainty of HOMA-IR is calculated from its components, i.e. glucose and insulin. To achieve an acceptable uncertainty of calculated tests, the uncertainty of each components should be lower than the desirable levels. In this study we evaluated the impact of uncertainty of HOMA-IR obtained by two different methods and instruments on clinical decision making.

We evaluated HOMA-IR in 64 patients with normoglycemic index with cut-off value 1.70. Glucose levels were measured by ADVIA 1800, and insulin levels were measured by two different systems, ADVIA Centaur XP (System A) and Immulite 2000 (System B). The uncertainty of HOMA was calculated from the uncertainty of its components and the patients results were expressed as HOMA-IR ± kU (k: 1.96 and U: uncertainty).

HOMA-IR values of 76% of patients from system A and 38% of patients from system were found to be higher than cut-off value. However the uncertainty of system A (± 0.5 ) was higher than the uncertainty of system B (± 0.2 ).

Although the HOMA-IRs of system A was higher than the system B, due to high level of uncertainty, the reliability of system A was lower than the system B. The reliability of calculated laboratory tests depend on the uncertainty of the measured components. Increasing uncertainty decrease the reliability of test results and the reliability of calculated laboratory tests should be evaluated prior to clinical decision making.

Microbiology

Abstract number 0333

Cutaneous Mycobacterium chelonae infection after mesotherapy treatment

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Introduction: In recent years there has been increasing interest in non-surgical cosmetic procedures. Mesotherapy is presented as an alternative procedure to surgery, which involves intradermal injection of small quantities of various substances that stimulate dermis and subcutaneous tissue. Despite the risk associated with these procedures is theoretically low, have been reported multiple cases of skin and soft tissue infections by rapidly growing non-tuberculous mycobacteria (NTM) secondary to these treatments.

Objective: We report a case of cutaneous Mycobacterium chelonae infection associated with mesotherapy, highlighting the contribution of the clinical microbiology laboratory.

Case Report: A 37-year-old woman presented with subcutaneous abscesses with abdominal location. The lesions had developed 3 weeks after mesotherapy injections for the purpose of cellulite reduction. Clinical samples for microbiological examination (direct and cultural exam) were obtained by needle aspiration. After 96 hours of incubation occurred the growth of small rough colonies in the blood agar plate, that were Gram positive bacilli and alcohol acid resistant when stained by Ziehl-Neelsen. The search for clinical information determined the cultural exam in Lowenstein Jensen, with growth of colonies suggestive of micobacteria in a week of incubation, identified as Mycobacteria chelonae by reverse hybridization PCR.
Conclusion: The growing demand for aesthetic treatments associated with poor regulation of clinics and products used in these alternative therapies caused an increase in infectious complications with NTM. The complexity of the diagnosis and treatment requires close communication between the clinician and the laboratory in order to guide the laboratory research on the etiological agent involved.

New technologies

Abstract number 0023

The organization of the transcriptional network in specific adipose tissues

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Fat depots vary in size, function, and potential contribution to metabolic disease risk. The basis for depot-specific differences in fatty acid handling, insulin sensitivity and endocrine function have been partly ascribed to intrinsic differences in gene expression. Using RNA-Seq, we have compared transcriptome changes in subcutaneous and three intra-abdominal white adipose tissue (WAT) depots as well as brown adipose tissue (BAT). To identify the pathways associated with depot-specific anatomical location, we used Weighted Gene Coexpression Network Analysis (WGCNA) to develop a systems-level view of transcriptional alterations within distinct adipose tissues from lean mice. Through WGCNA, we identified 9 co-expression modules that relate to fundamental cellular functions, such as cell communication, protein translation, cell adhesion, cell cycle, protein and DNA metabolism as well as immune response, suggesting that fundamental aspects of adipose diversity are produced by quantitative variation in basic metabolic processes. Importantly, we identify one module highly enriched for muscle-development genes that is present not only in BAT, but also in subcutaneous and retroperitoneal WAT, and absent in perigonadal and mesenteric WAT. These genes represent novel candidates that may regulate depot-specific adipose tissue development and function, which is supported by initial loss-of-function experiments in vitro. Together, the results provide a valuable resource to dissect gene regulatory mechanisms underlying the characteristic properties of anatomically distinct adipose tissues.

Abstract number 0148

New methodological solutions in hematology analyzer (Yumizen H500 - Horiba Medical) and clinical practice

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6 Warsaw, Horiba Medical, Poland

Introduction: Research of laboratory diagnostics includes issues: reducing the quantity of blood drawn from the patient, process automation, reducing a size of analyzer along with testing different methodology.

The aim of the study was to evaluate the analytical efficiency of new methodological solutions, used in hematology analyzer Yumizen H500, compared with the ADVIA 2120i in the assessment of peripheral cell blood counts (CBC) in oncology patients, including hematooncology subjects.

Methods: In 289 patients (174 women and 115 men; 20 to 82 years old), in the same venous blood sample, CBC determinations using two hematology analyzers were performed in parallel. The analyzer ADVIA 2120i uses optical technology combined with cytochemical reactions. Analyzer Yumizen H500 uses a combination of optical and impedance technology. Statistical analysis was performed using Statistica 10 version, taking into consideration the whole study population and 4 subgroups characterized by leukocytes count (WBC): WBC<1,00G/L; WBC1,00-3,99G/L; WBC4,00-10,00G/L, WBC>10,00G/L.

Results: Complete or almost complete correlations for routine CBC parameters achieved from the two analyzers (WBC r=0,999, RBC r=0,993, HGB r=0,989, HCT r=0,988, MCV r=0,966 and PLT r=0,992) were observed. However, the strength of the correlation for particular leukocyte populations was different: from almost full for WBC, lymphocytes and neutrophiles - in the entire study population and in each subgroup, to high or average - in subgroups of patients with leukopenia.

Conclusions: This evaluation shows that the smaller hematology analyzers, easier to use and cheaper could provide the high reliability of CBC results for general clinical practice.
Abstract number 0184

Evaluation of sediMAX as biological fluids analyzer

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Background: The last decade a new standard in the analysis of the elements present in the urine has been established. The most developed analyzer based on automated microscopy, SediMAX®, allows automatic recognition and counting of red blood cells (RBC), white blood cells (WBC), bacteria and other cells.

Aim of the study: To evaluate the use of SediMAX for the analysis of biological fluids, in comparison to Fuchs-Rosenthal counting chamber (gold standard).

Material and Methods: 59 samples of pleural and peritoneal fluids were analyzed using Fuchs-Rosenthal and SediMAX, and WBC and RBC were determined. We determined the correlation between both techniques for WBC, calculating sensitivity (SS), specificity (SP), positive predictive value (PPV), negative predictive value (NPV), positive and negatives likehood ratios (LR+, LR-) and Cohen’s kappa coefficient (κ). For RBC we determined the Pearson correlation coefficient (r) and the mean difference with a Bland-Altman plot.

Results: Correlation Sedimax/ Fuchs-Rosenthal for WBC: SS = 80.56%; SP = 95.65%; PPV = 75.86%; NPV = 96.67%; LR(+) = 18.53; LR(-)=0.20; κ = 0.73. Correlation Sedimax/Fuchs-Rosenthal for RBC: Bland-Altman plot mean difference = 357 (211 – 504) RBC; r = 0.903.

Conclusion: The degree of agreement between Sedimax and Fuchs-Rosenthal for WBC determination is substantial (κ = 0.73), there were very few false positive (SS = 80.56%, PPV = 95.65%), and what is more important in a screening test, only one false negative (SP = 95.65%, NPV = 96.67%).

The correlation between Sedimax and microscope for RBC counting is very good (r = 0.903), nevertheless the dispersion in results is high and mean difference between methods is elevated (357 RBC).

Abstract number 0196

Effect of oxidized low-density lipoprotein densification by ultrafiltration under nitrogen on level of its oxidative modification

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Objective: The aim of the study was to investigate whether in vitro densification of oxidized low-density lipoprotein (ox-LDL) by ultrafiltration under nitrogen influences the oxidation parameters of ox-LDL.

Methods: LDL fraction was isolated by ultracentrifugation from patients with familial hypercholesterolemia treated with LDL apheresis (n=5). LDL oxidation (at final concentration 1 mg/ml LDL protein) was performed in PBS containing 5 μM CuSO4 at 37°C for 18h. Ox-LDL were dialyzed against PBS containing 0.5 mM EDTA. Dialysis buffer (1500-fold sample volume) was changed three times over an 24h period. Oxidation parameters were estimated in native LDL and ox-LDL before and after densification. Thiobarbituric acid reactive substances assay (TBARS), free amino groups (FAG) evaluated by reaction with trinitrobenzene sulphonic acid (TNBSA) and conjugated dienes (CD) measured by absorption at 234 nm were used to estimate level of LDL oxidation. Protein concentration measured by modified Lowry method.

Results: Native LDL were characterized by low level of oxidative stress parameters: TBARS - 0.84±0.31 nmol/mg LDL protein; FAG - 436.2±53.8 nmol/mg LDL protein and CD - 0.478±0.013 AU. After oxidation, ox-LDL were concentrated about 5-fold by ultrafiltration under nitrogen and this process did not affect oxidative stress parameters: TBARS - 5.04±1.03 vs 4.77±1.04 nmol/mg LDL protein, p=0.186; FAG - 258.9±15.1 vs 254.1±16.8 nmol/mg LDL protein, p=0.481; CD - 1.274±0.071 vs 1.268±0.056 AU; p=0.568.

Conclusions: Densification of oxLDL by ultrafiltration under nitrogen does not affect oxidation level of ox-LDL thus may be used to concentrate of ox-LDL.

Abstract number 0215

Multicolor flow cytometry in modern diagnostics of acute leukemias

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Objective: The aim of the study was to evaluate the use of SediMAX for the analysis of biological fluids, in comparison to Fuchs-Rosenthal counting chamber (gold standard).

Material and Methods: 59 samples of pleural and peritoneal fluids were analyzed using Fuchs-Rosenthal and SediMAX, and WBC and RBC were determined. We determined the correlation between both techniques for WBC, calculating sensitivity (SS), specificity (SP), positive predictive value (PPV), negative predictive value (NPV), positive and negatives likehood ratios (LR+, LR-) and Cohen’s kappa coefficient (κ). For RBC we determined the Pearson correlation coefficient (r) and the mean difference with a Bland-Altman plot.

Results: Correlation Sedimax/ Fuchs-Rosenthal for WBC: SS = 80.56%; SP = 95.65%; PPV = 75.86%; NPV = 96.67%; LR(+) = 18.53; LR(-)=0.20; κ = 0.73. Correlation Sedimax/Fuchs-Rosenthal for RBC: Bland-Altman plot mean difference = 357 (211 – 504) RBC; r = 0.903.

Conclusion: The degree of agreement between Sedimax and Fuchs-Rosenthal for WBC determination is substantial (κ = 0.73), there were very few false positive (SS = 80.56%, PPV = 95.65%), and what is more important in a screening test, only one false negative (SP = 95.65%, NPV = 96.67%).

The correlation between Sedimax and microscope for RBC counting is very good (r = 0.903), nevertheless the dispersion in results is high and mean difference between methods is elevated (357 RBC).
Flow cytometry nowadays is an irreplaceable technique in diagnostic laboratory. It enables quick, relatively easy and cost-effective diagnostics of hematological diseases. Flow cytometric diagnostics has evolved for three decades and the most important changes include the increase in number of routinely used parameters (from 3 up to 8-10) and awareness of instrument performance control (quality assessment). Higher number of simultaneously assessed parameters reduces the number of tubes to process and acquire without increasing the costs. Additional advantages encompass the increased precision in cell phenotype assessment and better recognition of cell maturational stage. The use of multicolor diagnostic antibody panels enables to repeat leukemia lineage-specific antigens, forming the so-called panel backbone, leaving enough space for markers specifying the immunophenotype of leukemic blasts, important prognostic antigens or markers useful for minimal residual disease monitoring. Innovative software can merge many files and generate a single file containing the information on all markers used in the particular diagnostic approach. Based on this concept, one single case is stored as a single point, placed in n-dimensional space, where “n” means the number of parameters describing the point like coordinates. This approach enables to create databases of reference cases with confirmed diagnosis and subsequently to compare every newly diagnosed case against these defined subgroups, which significantly reduces the diagnosis time and opens the possibility of process automation. To obtain this, it is critically important to fulfill the quality assessment requirements, which is a prerequisite in modern diagnostics, not only based on flow cytometry.

Abstract number 0219

Verification of automated immunochemical fecal occult blood analyzer OC-SENSOR io

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Introduction: OC-SENSOR io is a automated immunochemical fecal occult blood analyzer. The measurements are based on a latex agglutination with polyclonal anti hemoglobin A0 antibodies. The aim of this study was to implement the analytical verification of the OC-SENSOR io (Eiken Chemical Co., Japan) analyzer to ensure reliable results of the laboratory test.

Materials and methods: Verification was performed using the CLSI procedure EP 15-A2. Control samples OC-Control LV1 and LV2 (Eiken) were used for five days in triplicate. Precision within the series was determined in 5 patients stool samples by 20 measurements at low concentration levels. The stability (N=12) was assessed at +4°C and +20°C during 21 days. Stool samples (N=179) assessed by the OC-SENSOR io (cut-off 50 ng/ml) and the results were compared with immunochromatographic test CoproHemoGnost (limit of detection 40 ng/ml).

Results: The coefficients of variation (CV) for repeatability were 6.0% for the LV1 (mean value 153 ng/ml), and 1.5% for the LV2 (mean value 469 ng/ml). Interprecision was 4.9%, and 1.6%, while overall laboratory precision was 6.9% and 2.0%. CV for the stools sample with mean value 50 ng/ml was 10.1%. The stability of the samples at two different temperatures didn’t show significant differences. Comparison of the methods showed the moderate strength of agreement (Kappa=0.493).

Conclusion: The OC-SENSOR io analyzer showed satisfying performances with regard to precision and stability of stool samples. Therefore, it enables application of quantitative data, as well as adjustment of sensitivity using the cut-off value.

Abstract number 0221

The determination of vitamin K1 and K1 2,3-epoxide in human serum/plasma by ultra performance convergence chromatography (UPC2) with LCMSMS

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The laboratory evaluation of vitamin K status is challenging. However, continued interest in roles that vitamin K play beyond that of coagulation, and in particular in bone and vascular health has led to an increase in demand for robust laboratory assays capable of processing large numbers of requests. For the past 20 years status has most commonly been determined through the measurement of phylloquinone (vitamin K1) in serum and plasma by HPLC with fluorescence detection. Inherently limitations include a requirement for long chromatographic run times and the need to chemically induce fluorescence.

We used Ultra Performance Convergence ChromatographyTM (UPC2) and a Xevo TQSmicro to rapidly determine endogenous levels of K1 [reference range 0.15–1.55μg/L] and the intra cellular metabolite vitamin K1 epoxide (K1O), [<0.12μg/L]. Baseline separation of K1, K1O...
and a deuterated form of vitamin K1 (K-d7) was achieved on the ACQUITY UPC2 HSS C18 column in 2.4 mins., with the total run time of 5.5 mins. under a gradient elution of a carbon dioxide and methanol mixture (1% to 40% methanol), isocratic solvent manager (ISM) 2% FA in methanol. Methanol calibration curves exhibited linearity in the range of 0.1μg/L for K1 and for K1O. The quantifier MRM transitions for K1, K1O and K-d7 were: 451.4→187.2, 467.6→307.5, and 458.4→194.1 m/z respectively. Using 1μl of serum extracts the LLOD was 0.1μg/L for K1 and K1O.

The developed UPC2 method appears suitable for rapid assessment of vitamin K status. It’s simple to perform and practical for routine K1 and K1O determination in serum.

Abstract number 0222

Study of blood collection and sample preparation for analysis of leukocyte cystine level by LC-MS/MS method

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Cystinosis is an autosomal recessive disorder characterized by accumulation of cystine in lysosomes throughout the body. In order to diagnose cystinosis and monitor treatment with cysteamine, adequate measurements of cystine concentrations in leukocytes and cultured fibroblasts are required. Previous methods reported for the measurement of cystine in leukocytes mainly include ion exchange chromatography, HPLC with fluorometric detection and cystine-binding protein assays. Nowadays, LC coupled with tandem mass spectrometry (LC-MS/MS) and ESI ionization, is becoming the most preferable approach, for better sensitivity and selectivity. Although several methods were developed for the determination of leukocyte cystine levels, to the best of our knowledge there is no study to examine the effect of blood collection and sample preparation type on this analysis. The aim of this study was to compare the influence of the protocol for the protein separation methods and blood collection on determination of leukocyte cystine levels by LC-MS/MS.

Three protein precipitation methods using different agents (sulfosalicylic acid, organic-solvent and trichloroacetic acid) have been evaluated in terms of sensitivity to delimit their application. Trichloroacetic acid was observed to be the best protein precipitation method for determination of leukocyte cystine level, according to the obtained limit of quantification and linear calibration ranges. The influence of blood collection tubes (heparin, EDTA and citrate) and leukocyte lysis methods including freeze-thaw, ultrasonic-bath and pellet-pestle motor on cystine analysis have been evaluated.

According to the results, blood-collection and sample pre-treatments have proved to be significant for the analysis of leukocyte cystine levels by LC–MS/MS.

Abstract number 0243

Development and validation of an ultra-performance liquid chromatographic-tandem mass spectrometry (UPLC-MS/MS) method for determination of cystine in leukocytes

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Cystinosis is a disorder characterized by accumulation of the amino acid cystine in lysosomes. Measurement of intracellular cystine in mixed leukocytes has been the standard method for both diagnosis of cystinosis, and for therapy monitoring. In this study, we aimed to develop and validate a selective, sensitive and rapid LC–MS/MS method for quantification of the cystine in Leukocytes using d6-deuterated cystine as an internal standard (IS). First we focused on organic solvent ratio (B1) in solvent system, formic acid concentration parameters for method optimization. We aimed to investigate effects of these parameters on analysis and developed the experimental technique for optimization of the method by using these parameters. In the developed method, ACQUITY UPLC BEH-C18, 2.7µm-2.1x50mm column was used as the stationary phase and 0.05% formic acid in acetonitrile/water 50/50% were used as the mobile phase. Injection volume was 2μL while flow rate was 0.2mL/minute. Mass-spectrums were determined with Waters-Xevo-TQD MS/MS system. 12%trichloroacetic acid was used as the protein-precipitation agent for leukocyte proteins. Retention times for cystine / d6-deuterated-cystine were 0.79 minutes. The method was fully validated in terms of selectivity, limits of detection(LOD) and quantification(LOQ), linearity, matrix effect, reproducibility, precision and accuracy. The developed method was validated in human leukocyte with a lower limit of detection 0.039 mM. A linear response function was established for the range of concentrations 0.078–25 mM (r > 0.995) for cystine. The precision(%CV) and accuracy results in five validation batches across five concentration levels were well within the acceptance limits.
Abstract number 0246

High-performance liquid chromatography for HbA1c in low-volume laboratories: analytical verification and method comparison study of Tosoh HLC-723GX analyzer

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Introduction: High-performance liquid chromatography (HPLC) determination of HbA1c has long been used as a reproducible and versatile analytical tool in clinical laboratories. However, the need for dedicated equipment and expertise limited its use to high-volume laboratories. Recently introduced user-friendly, fully automated benchtop HPLC analyzer (Tosoh HLC-723GX, Tosoh, Japan) enabled low-volume laboratories to provide equally reliable HbA1c results.

The aim of this study was to perform analytical verification of Tosoh HLC-723GX analyzer and compare the results with both the reference capillary electrophoresis (CE) and the immunoassay (IA) procedure.

Materials and Methods: The total imprecision of Tosoh HLC-723GX was verified in accordance with CLSI EP15-A2 protocol using commercial control materials (C-QC) and pooled human whole blood samples (HWB). Comparison of HbA1c results was performed on 146 samples (3.2-14.4% / 12-134 mmol/mol). HPLC-HbA1c results were compared to the automated CE (CE; MiniCap Flex Piercing, Sebia, France) and IA (IA; Tina-quant HbA1c Gen 2, Cobas Integra 400+ , Roche Diagnostics, USA) procedures, respectively.

Results: The total imprecision of HPLC-HbA1c was 1.25/1.91% (HbA1c = 4.8%/29 mmol/mol) and 0.51/0.63% (HbA1c = 97%/82 mmol/mol) in C-QC, and 0.51/0.66% (HbA1c = 6.5%/47 mmol/mol) and 0.65/0.87% (HbA1c = 10.7%/94 mmol/mol) in HWB samples, respectively. Bland-Altman analysis did not reveal any deviation of the results between HPLC and CE: 0.0% (95%Cl: -0.02927 to 0.02653%), while the average HbA1c deviation between HPLC and IA was -0.07% (95%CI: -0.1039 to -0.02765).

Conclusion: The performance of Tosoh HLC-723GX met the quality criteria for reliable clinical use, thereby representing a plausible analytical choice for HbA1c measurement in low-volume laboratories.

Abstract number 0263

Verification and implementation of automated peripheral blood smear analyzer Vision Hema Assist to the routine laboratory work

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Introduction: The new automated bloods smear analyzer Vision Hema Assist, West Medica has been introduced to the routine laboratory. The aim of this study was performance evaluation as well as interchangeability of its results with the results obtained by manual microscopy for the clinical practice.

Methods: We evaluated 71 peripheral blood smears, of which 50 were neonatal samples. Complete blood count was performed by hematology analyzer Sysmex XE5000 using analytical methods accredited according to ISO 15189 and controlled by external quality assurance programme, blood smears were made and MGG stained at the same time. Smears were examined manually and by automated analyzer which results were checked and reclassified if necessary by a staff with abundant morphology experience. Results of relative leukocytes differential count from automated analyzer were compared with results from hematology analyzer and ones gained by manual microscopy.

Results: Mean bias for segmented neutrophil granulocytes, lymphocytes, monocytes, eosinophilic granulocytes, basophilic granulocytes, for both comparisons was less than 2%, 0.5%, 3%, 0.5%, 0.3%, respectively. For myelocytes, metamyelocytes and band neutrophils only automated and manual microscopy results were compared and mean bias was less than 0.1%, 0.1%, 0.7%, respectively. According to Passing-Bablok regression constant and proportional errors were found only for segmented neutrophils (y = 5.991(0,546-10,000) + 0.889(0,800-0,983)x) caused by patients with neutrophilia and neutropenia where this difference is clinically insignificant.

Conclusions: Evaluation has shown excellent comparison of obtained results. Therefore, manual and automated blood smear analysis can be used interchangeably for the clinical purposes. Still, despite great performance of automated analyzer, analysts experience is essential.

Abstract number 0269

Plasma cells characterization by Beckman Coulter Unicell DxH 800 hematology analyzer

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Plasma cells are antibody producing cells derived from B-lymphocytes, residing mostly in lymphoid tissue. Presence of plasma cells in peripheral blood is sometimes associated with malignant processes of lymphoid tissue, but also can be found in patients suffering from some infectious diseases (varicella, infectious mononucleosis, toxoplasmosis, rubella). During several past years we noticed that Beckman Coulter Unicell DxH 800 hematology analyzer, in presence of plasma cells in peripheral blood, produces flags referred to malignant cells presence. The aim of this work was finding all possible flags produced in presence of 3 or more plasma cells/100 leukocytes (proved by microscopic procedure performed by two independent laboratory workers), in the period of last six months. Our results show that out of 29 samples that fulfilled the above criteria the most frequent flags were: 1) MO Blast (8 patients), 2) Variant lymphocytes (6 patients), 3) Variant lymphocytes/MO Blast (5 patients), 4) No flag (4 patients), 5) Variant lymphocytes/MO Blast/Ly Blast (2 patients), 6) Variant lymphocytes/Ly Blast (2 patients), 7) Mo Blast/Ne Blast (1 patient). Experienced laboratory worker may expect that besides flags frequently associated with malignant hematology disease, plasma cells could also be detected. However, all those flags should be confirmed by microscopic examination to exclude malignant cells.

Abstract number 0342

SDS electrophoresis as a qualitative and semiquantitative tool for evaluation of proteinuria

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Proteinuria is one of the clinical laboratory findings with serious clinical consequences. However, there are several different mechanisms leading to the presence of protein in urine:
1. abnormal transglomerular passage of proteins due to increased permeability of glomerular membrane;
2. impaired reabsorption by the epithelial cells of the proximal tubules;
3. production of large amounts of low molecular weight proteins during diseases without initial kidney involvement (multiple myeloma, rhabdomyolysis). Determination of the initial cause of proteinuria is critical for further diagnostics and treatment. Main mechanisms of proteinuria may be determined by analysis of the molecular weight profile of excreted proteins.

The aim of the study was an evaluation of the different SDS electrophoretic variants (different gel concentrations) for the evaluation of proteinuria.

Results: SDS electrophoresis stained with popular CBB-G stain has high sensitivity down to 5mg/l/protein band, covering normal and microalbuminuria concentration ranges. Intensity of the protein band enables semiquantitative determination of particular proteins (albumin, free light chains, uromodulin). Gels with 12% acrylamide concentration enable detection of albuminuria and assessment of selectivity of glomerular proteinuria. Gels with higher acrylamide concentrations (16%) are very well suited for investigation of tubular proteinuria and overflow free light chains proteinuria. Best results were obtained by commercial 4-20% gradient gel systems.

Conclusion: SDS electrophoresis is a sensitive tool for determination of molecular mass dependent profile of urine proteins. It enables determination of the mechanism of proteinuria and gives initial semiquantitative results concerning concentrations of the selected marker proteins.

Abstract number 0343

Evaluation of the performance of a new automated system (INDEXOR ®) in the archive unit of a clinical laboratory

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Aims: In clinical laboratories the main source of error is the pre-examination phase which accounts for 50-75% of all laboratory error. Sample identification, and correct knowledge of the place of a sample will decrease pre-examination errors. An automated system (INDEXOR ®) has been developed to track and organize the locations of samples within and outside of clinical laboratories. We aimed to evaluate the performance of INDEXOR and compare it with a manual sample-finding procedure within the archiving unit of laboratory.

Methods: We used five different groups and in the first group, we tried to find only a one sample and in the fifth group we tried to find five different samples in the archive. We determined a cut-off time for each group: 1 min for the first group (1 min to find one sample) and 5 min for the fifth group (5 min to find five samples). If the time taken to find a sample was greater than its cut-off time, the search was considered a defect. We converted all defects to Sigma Metrics.
Results: The Sigma Metrics for all INDEXOR groups were greater than 6, whereas the Sigma Metrics of all manual groups were lower than 1. In comparison with the manual procedure and based on the chosen cut-off values, the performance of INDEXOR was excellent. However, the manual procedure was completely unacceptable.

Conclusion: In clinical laboratories, INDEXOR is a user-friendly, cost effective and time-saving system that increases the work flow and efficiency significantly.

Protein assays

Abstract number 0077

Unexpected migration of free light chains in urinary protein electrophoresis

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Urinary protein electrophoresis (UPE) is frequently used to detect abnormal free light chains (FLC) secretion in urines. Urinary FLC can be secreted as monomer or dimer. When Sodium Dodecyl Sulfate (SDS) is used before migration, as in the Hydragel Proteinuria method from Sebia, FLC migrate according to their molecular weight, and usually are revealed as a pattern of two bands. We report two clinical cases of monoclonal IgG kappa myeloma presenting unexpected migration of FLC on the UPE gel.

The first patient presented a particular band in the beta2microglobulin migration area UPE gel although urinary beta2microglobulin was not detectable by immunoassay. The second patient presented two bands firstly assigned to albumin and transferrin, whereas urinary albumin concentration was low.

We used another UPE method without SDS (Hydragel HR, Sebia) that confirmed there were unusual migrations of FLC. Any band appeared in the betaglobulins area in the first case. In the second case a very light band was observed in the albumin migration area. Moreover, the addition of a depolymerizing mix to the second patient urines led to a strong decrease in the intensity of the supposed albumin and transferrin bands combined with an increasing intensity of the monomeric FLC migration area.

Unexpected FLC migrations presented here might be the result of damage or polymerization of FLC in the first and second case, respectively. The main goal of these clinical cases is to keep in mind that interpretation of protein electrophoresis can be difficult in complex fluids such as urines.

Abstract number 0104

Intact immunoglobulin and free light chain gammopathies. Timelines and relationship to infection after heart transplantation

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Background: Solid organ transplant recipients are at increased risk of lymphoproliferative disorders, immunoglobulin abnormalities, and infection due to immunosuppressant therapy.

Aim: To analyse the relationship among intact immunoglobulins, free light chains (FLC), time, and infection after heart transplantation (HTX) in a pilot study.

Methods: Total of 96 patients (81 men, 15 women: age 21-74 years) after HTX (2010-2012) were tested. Blood samples were obtained before, +9, +18 and +24 months post-HTX. Immunosuppressant dosage was highest in first 9 months, reduced thereafter. Infection was defined as symptoms and positive leukocytes or CRP. The FLC and heavy light chain-pairs (HLC) were measured using Binding Site kits on SPA analyzer.

Results: Significant differences (p<0.001, Friedman test) were found during follow-up with lowest concentrations +9 months for IgA kappa (30% from basal value), IgA lambda (29%), IgG kappa (29%), IgG lambda (-6%), FLC kappa (-39%) and FLC lambda (-28%) and steady rise up to 24 months. No significant dynamics were found for IgM kappa and lambda. Patients with infection had lower FLC kappa (p=0.008) and lambda (p=0.028) as well as HLC IgG kappa (p=0.0034) in 9th month.

Conclusions: Significant changes in HLC and FLC were found after HTX during a 2 year follow-up, possibly as a result of immunosuppressant dosage changes. Lowest concentrations of FLC kappa, FLC lambda and HLC IgG kappa were found 9 months after HTX in infected patients. Further study is needed to elucidate these changes. Affiliations: Ministry of Health of the Czech Republic support (Grant number: AZV MZ 15-27579A).
Abstract number 0123

**An automated particle-enhanced immune turbidimetric test for urinary orosomucoid: validation and clinical usage**

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Orosomucoid or alpha-1 acid glycoprotein is an acute-phase protein with a molecular mass of 42-43 kDa. It is a normal component of urinary proteins. Regarding inflammatory disorders, it has been suggested that urinary orosomucoid (u-ORM) may provide information on the severity of these diseases. However, the clinical benefit of u-ORM determination has not been established yet. We would like to demonstrate the validation of an automated method for u-ORM measurements. Furthermore, our aim was to investigate u-ORM levels in acute and chronic inflammatory diseases.

A particle-enhanced immune turbidimetric assay was adapted for a Cobas 8000/c502 analyzer. Spot urine samples of healthy individuals (n=72), patients with Crohn’s disease (n=28) and septic patients (n=30) were analyzed. We expressed our data in u-ORM/creatinine ratios (mg/mmol) and in u-ORM/urinary total protein proportion (%).

The detection limit was determined to be 0.02 mg/L. The intra- and inter-assay imprecision CV% and also the inaccuracy were found to be less than 5%. Reference values for u-ORM/creatinine ratio were established to be 0.08 (0.01-0.24) mg/mmol [median (2.5-97.5 percentiles)]. Compared to controls, u-ORM/creatinine ratios showed a 5-fold elevation in Crohn’s disease and approximately 230-fold elevation in sepsis (p<0.001) while serum ORM values increased only moderately (at about 2-fold).

We set up a highly sensitive, precise and accurate turbidimetric approach for ORM determination in urine. Our fully automated assay is ideal for routine utilization and our findings support that u-ORM measurements can be a novel laboratory test for diagnosis and monitoring of inflammatory processes.

Abstract number 0129

**Synergistic, predictive protein markers in sepsis: serum Gc globulin and gelsolin**

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We focused on investigation of the predictive value of two actin binding proteins, serum group-specific component (Gc globulin) and gelsolin (GSN) in SIRS and in sepsis. Serum samples of 12 SIRS and 32 septic patients from our university’s Intensive Care Unit were obtained on day 1, 3, 5, after clinical diagnosis (ethical permission: 4327.316-2900/KK15/2011). The control group consisted of 28 ophthalmologic patients. Serum Gc globulin measurements were performed by an immune turbidimetric assay on Cobas 8000/c502 analyzer (Roche), GSN levels were estimated by quantitative chemiluminescence Western blot. hsCRP, procalcitonin (PCT) levels were determined by automated routine laboratory techniques. Data are expressed as medians. SIRS and septic patients exhibited significantly lower first-day Gc globulin (SIRS: 195.5 mg/L; sepsis: 158.2 mg/L; p<0.01) and GSN levels (SIRS: 31.6 mg/L; sepsis: 15.2 mg/L; p=0.001) compared to controls (Gc: 341.2 mg/L; GSN: 65.1 mg/L). First-day GSN levels were significantly higher (p<0.01) in SIRS than in sepsis. Septic patients with 7-day mortality showed lower (p<0.05) first-day Gc globulin (46.7 mg/L) and GSN concentrations (8.9 mg/L) than survivors (Gc: 194.9 mg/L; GSN: 16.9 mg/L). Gc globulin had a higher AUC-ROC (0.79; p<0.001) for predicting 7-day mortality in sepsis compared to PCT (0.75; p<0.05) while GSN showed similar AUC-ROC values (0.74; p<0.05). Gc globulin also performed better (AUC: 0.85; p<0.01) in predicting 3-day mortality of sepsis than PCT (0.80; p<0.05). Both Gc globulin and GSN are promising predictive markers of sepsis in the intensive care unit.
Thyroid disorders

Abstract number 0227

TT4 vs. FT4 in laboratory practice

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Thyroid function tests are among the most common requirements in the laboratory. According to the guidelines, thyrotropin (TSH) is recommended as a first test for thyroid function. If the TSH is outside the RI it’s recommended to make FT4. FT4 is the biologically active fraction of the hormone that doesn’t depend on the concentration of binding proteins, and has a better detection of the discriminative power of the hypothyroid or hyperthyroidism in relation to TT4. However, severe nonthyroid diseases, the treatment of heparin and some medications, and fasting may increase FT4 with no indications of increased secretion of T4.

The aim of this study was to measure the concentration of TT4 in patients with increased FT4 while normal TSH, and in patients with normal TSH and FT4.

The concentrations of TSH, FT4 and TT4 were measured in serum samples (n=78) by chemiluminescent microparticle immunoassay (CMIA) on UniCel Dxl600 (Beckman Coulter Inc., Brea, USA). The results were processed by MedCalc (MedCalc Software, Mariakerke, Belgium).

The analysis shows that only 5 patients (11%) of the total 44 have elevated TT4 with elevated FT4 and normal TSH. Correlation coefficient between TT4 and FT4 for these subjects was 0.68. Others have TT4 within the RI, in accordance with TSH and clinical data. The results showed that certain factors can influence the concentration of FT4 and so result in nonconformance with TSH values and the clinical data. In order to obtain more accurate indication of the thyroid function in these patients is needed to measure and TT4.

Abstract number 0260

Endothelial cell markers in patients with hypothyroidism

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Aim: To evaluate the effect of hypothyroidism on endothelial cell activation or damage. Methods: Thirty patients with clinical symptoms of hypothyroidism were investigated: 15 with overt hypothyroidism and 15 with mild hypothyroidism, among them were 26 women and 4 men and mean age was 44 ± 14 years. The control group of 30 subjects without clinical symptoms of hypothyroidism consisted of 28 women and 2 men and mean age was 45 ± 10 years. Serum sE-selectin and sP-selectin concentrations (endothelial activation markers) and plasma von Willebrand Factor concentration (vWF, endothelial damage marker) were determined with the ELISA method. The serum interleukin-6 concentration (IL 6, pro-inflammatory cytokine) was determined using ELISA method and the C-reactive protein concentration (hsCRP) was determined using latex-enhanced immunonephelometric assay. Results: Patients with hypothyroidism were characterized by significantly higher IL-6 concentration than the control group (p < 0.01). The concentrations of sE-selectin, sP-selectin, von Willebrand Factor and hsCRP did not differ significantly between hypothyroid patients and the control group. The vWF concentration in patients with hypothyroidism correlated inversely with the TSH concentration (r = -0.3974) and positively with the fT3 concentration (r = 0.4278). There was a positive correlation between sE-selectin concentration and hsCRP concentration (r = 0.4248). Conclusions: Chronic inflammatory processes accompanied hypothyroidism enhance endothelial cell activation, but the vWF concentration decrease, directly related to the severity of hypothyroidism, weakens the prothrombotic processes on endothelial surface.

Abstract number 0278

The association of red cell distribution width with cardiovascular risk factors after kidney transplantation

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