2nd European Joint Congress of EFLM and UEMS "Laboratory Medicine at the Clinical Interface"

7th Congress of the Croatian Society for Medical Biochemistry and Laboratory medicine (CSMBLM)

Hotel „Rixos"
Dubrovnik, Croatia
10-13 October 2012

Organizers:

European Federation of Clinical Chemistry and Laboratory Medicine (EFLM, formerly EFCC)
European Union of Medical Specialists Board of Medical Biopathology (UEMS LM-SMB)
Croatian Society for Medical Biochemistry and Laboratory Medicine (CSMBLM)

Auspices:

International Federation of Clinical Chemistry and Laboratory Medicine (IFCC)
Bernadette Vergnaud (France), Member of the European Parliament

COMMITTEES

Scientific Committee of the 2nd EFLM-UEMS Congress

Gerhard Zlabinger (UEMS, Austria), Chair
Gabor Kovacs (EFLM, Hungary)
Sverre Sandberg (EFLM, Norway)
Ian Frayling (UEMS, United Kingdom)
Ilkka Mononen (UEMS, Finland)
Ana-Maria Simundic (CSMBLM, Croatia)
Elizabeta Topic (CSMBLM, Croatia)

Scientific Committee of the 7th CSMBLM Congress

Tihana Zanic Grubisic (Zagreb, Croatia), Chair
Ivana Cepelak (Zagreb, Croatia)
Zlata Flegar Mestric (Zagreb, Croatia)
Lada Rumora (Zagreb, Croatia)
Ilza Salamunic (Split, Croatia)
Ines Vukasovic (Zagreb, Croatia)
Renata Zadro (Zagreb, Croatia)

Congress Organizing Committee

Ana-Maria Simundic (CSMBLM, Croatia), Chair
Lora Dukic (CSMBLM, Croatia)
Stefica Dvornik (CSMBLM, Croatia)
Manuela Lovric (CSMBLM, Croatia)
Sanja Marevic (CSMBLM, Croatia)
Nora Nikolac (CSMBLM, Croatia)
Andrea Saracevic (CSMBLM, Croatia)

Vic Blaton (EFLM, Belgium)
Lena Norlund (UEMS, Sweden)

Plenary lectures

PL1 - Preventive, predictive and personalized medicine - where are we?

PL1 - 1

European strategies, new guidelines & standardisation in predictive, preventive & personalised medicine

Golubnitschaja Olga

European Association for Predictive, Preventive and Personalised Medicine, EPMA, Radiological Clinic, Friedrich-Wilhelms-University of Bonn, Bonn, Germany

Corresponding author: olga.golubnitschaja@ukb.uni-bonn.de

Predictive, preventive & personalised medicine is a new strategy in healthcare aiming at application of innovative biotechnologies in the prediction of human pathologies, the development of timely prevention and individualised therapy-planning. Predictive diagnostics is considered as a reliable navigation system for targeted preventive measures and consequent development of treatment approaches tailored to the patient. Of paramount importance is communication among professionals – medical doctors, biotechnologists, computer-scientists, healthcare providers, policy-makers, educators, who are obligatorily involved in the paradigm change from delayed interventional to predictive medicine. This concept is considered as medicine of future. New strategies which EPMA represents for further consideration at the EU-Commission, the European Parliament, WHO and UNO are elaborated by the consortium of the world-leading professionals (Europe-unrestricted). The EPMA Mission and Objectives in the field of Predictive, Preventive & Personalized Medicine (PPPM) have been introduced to the Organisation of United Nations. The participants of the meeting agreed that the paradigm change can be achieved only by coordinated measures well-focused on solving the accumulating problems in healthcare and the concomitant economical burden that societies across the globe are facing more and more.

PL1 - 2

Recent technology towards predictive, preventive and personalized medicine

Ferrari Maurizio

Vita-Salute San Raffaele University, San Raffaele Scientific Institute, Genomic Unit for the Diagnosis of Human Pathologies, Center for Translational Genomics and Bioinformatics, Milano, Italy

Corresponding author: ferrari.maurizio@hsr.it

Personalized medicine, which simply means selection of treatment best suited for an individual, involves integration and translation of several new technologies in clinical care of patients.

Researchers have discovered hundreds of genes that harbour variations contributing to human illness, identified genetic variability in patients' responses to dozens of treatments, and begun to target the molecular causes of some diseases. In addition, scientists are developing and using diagnostic tests based on genetics or other molecular mechanisms to better predict patients' responses to targeted therapy.

Advances in DNA analysis to develop methods, which are increasingly specific, sensitive, fast, simple, automatable, and cost-effective, are considered paramount. These demands are currently driving the rapid evolution of a diverse range of newer technologies.

For the future of genomics is demanding the rapid evolution of miniaturization (nanotechnogy) and high-throughput genotyping technologies (next generation sequencing) toward increased speed and reduced cost.

The success of personalized medicine depends on having accurate diagnostic tests that identify patients who can benefit from targeted therapies. Within the past few years, a growing number of businesses have begun to offer direct to consumer genetic tests. These tests are designed to help individuals better understand their genetic predisposition for a given health condition.

Another important step will be expanding efforts to develop tissue banks containing specimens along with information linking them to clinical outcomes.

In this arena Laboratory Medicine should play a major role.

PL2 - Laboratory medicine online

PL1 – 1

Health literacy on laboratory tests

Abstract not provided.

PL2 - 2

Mobile technology and laboratory information services

Soini Esa

Mylab Corp., CEO, Tampere, Finland

Corresponding author: esa.soini@mylab.fi

Throughout the history there have been several successful technology breakthroughs that have had a global impact. The latest addition to these revolutionary technologies is the mobile communication. The wireless mobile technology sets us free from the limitations of time and space.

In principle mobile technology means methods and equipment that use electromagnetic signals for the wireless communication. The mobile technology is mainly based on radio waves invented by Guglielmo Marconi at the end of the 19th century. Because electromagnetic spectrum is a scarce resource there is a need for very strict communication standards. We have the Global Positioning System (GPS) and its European versions for worldwide accurate navigation. General wireless point-to-point communication utilizes various digital cellular standards like GSM, GPRS, WCDMA, and LTE. The best method for mobile Internet usage is so called Wireless Local Area Network (WLAN). Finally we have different technologies for very short distance communication like Bluetooth, RFID, and Near Field Communication (NFC).

Internet and the above mentioned mobile technologies form a solid infrastructure that can be used via personal digital devices. Examples of these are laptop computers, tablet computers, and smart phones. Many mobile devices are capable to utilize almost all of the previously mentioned communication standards. Large companies are creating mobile ecosystems around these technologies.

Clinical laboratories can now provide comprehensive information services based on mobile technology. Almost every phase of the laboratory process from specimen collection to results reporting will be in the future based on mobile technology.

PL3 - How to approach malnutrition from laboratory point of view?

PL3 – 1

Failure to thrive in childhood and the laboratory

Abstract not provided.

PL3 – 2

Clinical biochemistry in nutrition practice

Alastair Forbes

UCL, London, United Kingdom

Corresponding author: a.forbes@ucl.ac.uk

Nutrition screening is of considerable value in identifying patients with malnutrition or at high risk of malnutrition, and the most successful screening tools rely on simple clinical tools without the need for venesection or urine collection

(and therefore avoidany delay beyond the time of interview).

Nutritional assessment is a more detailed process that will normally include a set of anthropometric measurements, and may include study of body composition (such as with bioelectrical impedance or DEXA scanning), and indirect calorimetry to complement standard predictive equations (such as Harris-Benedict) for the determination of energy requirements, as well as a professional opinion of nutritional status. It is unusual for these assessments to include biochemical parameters of necessity, or for the conclusion to incorporate biochemical data, although most patients will have had simple routine laboratory assays performed.

Those outside the clinical nutrition field often consider that the serum albumin is a marker of malnutrition, but there is very little evidence in favour of this assumption. There is no doubt that a low albumin is strongly predictive of a poor outcome, and there is therefore a positive correlation between low albumin and malnutrition, but it is not now considered to be of a causal nature. A similar conclusion can be attached to many other biochemical analyses, and none of the major clinical nutrition guidelines recommends their use in initial assessment. In summary we might usefully conclude that biochemical analysis plays little role in macronutrient assessment.

Nonetheless patients with malnutrition frequently have many biochemical deficiencies and in principle it would be good practice for clinicians to ensure that micronutrient status is comprehensively assessed. The difficulties here are that the assays for many micronutrients are not readily available, and because the interpretation of assay results is often neither simple nor transparent. This assessment will therefore often be limited to the measurement of a few key vitamins (e.g. folate, vitamins B12 and D) and elements (e.g. Mg, Se, Zn) and to a few surrogates (such as coagulation status for vitamin K).

Accordingly most of the interface between the clinical biochemist and the clinical nutritionist will be in management and monitoring of patients on artificial nutrition support and particularly those on parenteral nutrition (PN). Conventional hepatic and renal biochemistry will generally be monitored closely in the early days of PN, permitting adjustment and optimisation of volume and electrolyte load. Deteriorating liver function is rare in good hands and abnormalities much more often reflect underlying disease such as sepsis. Correction of micronutrient deficiency will also be monitored but this is not straightforward given the uncertainties of correct intravenous replacement and the availability of some micronutrients only in composite fixed dose formats.

Special and challenging situations such as the management of systemic acidosis or apparently intractable excess or deficiency of certain electrolytes provide the territory for productive liaison between the biochemical and nutritional disciplines.

S0 - Pre-congress CSMBLM symposium: External Quality Assessment for medical-biochemistry laboratories in Croatia

S0 - 1

Interlaboratory comparisons in laboratory medicine - implementing the requirements of HRN EN ISO/IEC

Flegar-Meštrić Zlata

Merkur University Hospital, Institute of Clinical Chemistry and Laboratory Medicine, Zagreb, Croatia

Corresponding author: zlata.mestric@gmail.com

The Croatian Society for Medical Biochemistry and Laboratory Medicine has a very long tradition in organizing external quality assessments (EQA) for medical biochemistry laboratories, therefore, in 2012, CROQALM – the Croatian centre for quality assessment in laboratory medicine was established. One of the main tasks of CROQALM is to apply the requirements of the international and European standard for accreditation of proficiency testing providers adopted in the Republic of Croatia as the Croatian Standard HRN EN ISO/IEC 17043 "Conformity assessment – General requirements for proficiency testing". Standard represents internationally harmonised "general requirements" for the accreditation of proficiency testing (PT) providers and requires defining the scope and specifying the requirements concerning the planning, design, testing, distribution, evaluation and reporting of PT/EQA results. In order to support implementation of ISO 17043, Croatian Accreditation Agency, as a national accreditation body, has established the Working Group for Interlaboratory Comparisons which works on PT/EQA regulations and guidelines. CROQALM subcontracts with commercial suppliers providing commutable, stable and homogenous material of high quality for various rounds and conducts schemes for the clinical chemistry, laboratory haematology and coagulation, and in the post-analytical phase, evaluation of laboratory reports. For the purpose of professional supervision, the Croatian Chamber of Medical Biochemists requests to be directly informed on laboratory participation in EQA schemes and achieved results. Therefore, according to the requirement of ISO 17043 regarding the confidentiality of all information supplied by a participant to the PT/EQA provider, CROQALM inform the participants of this action, in advance and in writing.

S0 - 2

Statistical evaluation of proficiency testing scheme results in laboratory medicine

Gašljević Višnja

Hrvatsko mjeriteljsko društvo, HMD, Zagreb, Croatia

Corresponding author: visnja.gasljevic@hmd.hr

The primary aim of proficiency testing is to allow laboratories to monitor and improve the quality of their routine measurements. One of the basic elements in every proficiency testing is the evaluation of the performance (score) of each participant. The most common scoring system is the z-score. Two critical steps in performance evaluation are: specifying the assigned value and setting the standard deviation for proficiency assessment. These influence the scores that the participant receives directly and therefore also the way they interpret their performance in the scheme. There are a number of different approaches to obtaining them, each with its advantages and disadvantages. Preferred statistical techniques have been described in the ISO 13528, although other valid approaches can be used (ISO 5725, median and NIQR, fit-for purpose criterion) as long as they are statistically valid and are fully described to participants. Special attention should be paid on data distribution, like presence of outliers and data asymmetry and number of the participants.

S0 - 3

Benefits of the new EQA software for medical laboratories in Croatia

Radišić Biljak Vanja

Merkur University Hospital, Institute of Clinical Chemistry and Laboratory Medicine, Zagreb, Croatia

Corresponding author: Vanja.Radisic.Biljak@idb.hr

A significant breakthrough in the acceptance and processing of results of Croatian external quality assessment scheme (EQAS) occurred in 2011 with the introduction of a new software: INlab2*QALM– application for quality assessment in laboratory medicine, developed by Croatian software company, IN2 Ltd. Software was accompanied by illustrative and comprehensive easy-to-use handbooks. Through software module for the implementation of Web access for participants great advantage was achieved in the acceptance of results that was fully implemented through an electronic form, unlike the paper registers used in previous years. Once entered, the settings for the methods, analyzers and reagents will remain available in each subsequent cycle until the participant does not modify them. Fast processing of results is provided through the software module for the administrator and scheme coordinators. Complete data processing can be finished within 2 weeks which is a remarkable improvement over the manual entry of results in previous software. With a very open cooperation among participants and EQAS administrator two cycles of EQAS were completed in 2011 using INlab2*QALM application. The success was very high, 98% of laboratories in cycle 2/2011 and 99% of laboratories in cycle 3/2011 have successfully uploaded their results through a web module and received online certificates of participation. The new functionalities were introduced in the web module in the beginning of 2012 - online application for participation in the EQAS followed by invoices and notifications to users. All laboratories (100%) managed to register for 2012. through web application module.

S0 - 4

Evaluating EQA results in general medical biochemistry - standardization of creatinine measurements

Perkov Sonja

University Hospital Merkur, Institute of Clinical Chemistry and Laboratory Medicine, Zagreb, Croatia

Corresponding author: sonja.perkov@gmail.com

The standardization of creatinine measurement is critical to promote early detection and management of chronic kidney disease and is mandatory before the routine implementation of the estimated glomerular filtration rate in clinical practice. Actual results for serum creatinine measurement in Croatia were compared with the current recommendations based on creatinine measurements traceable to isotope dilution mass spectrometry (IDMS) reference method within the national External Quality Assessment (EQA) Scheme conducted through Croatian Centre for Quality Assessment in Laboratory Medicine (CROQALM). The results of the cycle 2/2011, 3/2011 and 1/2012 from 187 medical biochemistry laboratories were evaluated. The uncompensated kinetic Jaffe method traceable to National Institute of Standards and Technology (NIST) Standard Reference Material (SRM) 909b level 2 is most widely used in medical biochemistry laboratories (80%, 73% and 68% of participants in the survey 2/2011, 3/2011 and 1/2012, respectively). Only 17%, 24% and 29% laboratories in the survey 2/2011, 3/2011 and 1/2012 used standardized creatinine methods traceable to the IDMS method and SRM 967. The interlaboratory SD for compensated kinetic Jaffe method group, traceable to the IDMS method and SRM 967 was very high: 12.1 µmol/L at a creatinine concentration of 98.8 µmol/L (12%), 19.3 µmol/L at a creatinine concentration of 204 µmol/L (9%) and 28.6 µmol/L at a creatinine concentration of 495.8 µmol/L (6%). One of the main goals of the Croatian EQA program in 2012 is to promote laboratories to implement standardized method and reduce the inter-laboratory variability which affects the total error for creatinine measurement.

S0 - 5

Evaluating participant performance in hormone analysis (TSH)

Đurić Koraljka

Croatian Society of Medical Biochemists, Croatian EQA Committee, Zagreb, Croatia

Corresponding author: koraljka.duric@sunce.hr

Background: TSH measurement has a critical role for detecting thyroid dysfunction. Although the immunoassays for TSH had remarkably improved, their reliability depends on differences in analytical sensibility and performance.

Materials and methods: Croatian EQA results for TSH were analysed for 2008-2011 period. Control samples used for EQA were commercial control materials. Results were evaluated according to analytical methods/instruments except when the number of participants was less than 7 in particular analytical groups. Overall analytical performance was evaluated according to the biological variation.

Results: There is constant increase in number of participants for TSH in Croatian EQA programme from 2008 to 2011 (45, 56, 59 and 62; respectively). Unfortunately, the number of different analytical methods according to manufacturer stayed high (12 in average). The application of defined quality specifications was possible for only 2 analytical methods in 2008-2009 and 3 in 2010-2011. This is equivalent to 25/45 (56%) of laboratories evaluated for TSH according to analytical method in 2008, 29/56 (52%) in 2009, 39/59 (66%) in 2010 and 41/62 (66%) in 2012, respectively. Evaluation for other participants was not method specific due to statistically inadequate number of reported results. EQA results for TSH showed overall variability (all analytical methods/instruments, expressed as coefficient of variation) to be 13.7% in 2008; 14.2% in 2009; 12.3% in 2010 and 14.5% in 2011.

Conclusions: Significant heterogeneity of immunochemical analytical methods and statistically inadequate number of results for most of them resulted in relatively high but still acceptable overall variability in Croatian EQA surveys for TSH.

S0 - 6

The role of EQA in implementation of the HbA1c global harmonization in Croatia

Vučić Lovrenčić Marijana

Merkur Teaching Hospital, Institute of Clinical Chemistry and Laboratory Medicine, Zagreb, Croatia

Corresponding author: vucic@idb.hr

Harmonization of the HbA1c results has been an ultimate challenge for health-care professionals involved in diabetes care worldwide. Long-term activities in the field resulted into the Global Consensus on the Standardization of HbA1c (2010), with dual reporting units: SI (mmol/mol) and NGSP (%) and IFCC reference system as a standard.

In Croatia, a dedicated module for HbA1c was established within External Quality Assessment Scheme (EQAS) under the auspices of the Croatian Society of Medical Biochemists in 2005, with a primary goal to improve analytical quality and provide data for inter-laboratory comparisons. After a total of 13 EQAS-cycles, HbA1c testing in Croatia considerably improved in both quantity and within-laboratory precision (N = 53 *vs*. N = 27 laboratories, and CV = 5.4% *vs*. CV = 9.52% in 2012 and 2005, respectively).

A pilot-scheme for dual reporting, in accord to the Global Consensus, was organized within regular EQAS-cycle in December 2010. Participating laboratories (N = 48) were provided with a modified result-form, covering both units, and a short explanation of the Global Consensus goals in Croatian language, together with HbA1c conversion table. Results of a pilot-scheme revealed an excellent compliance to the cycle-specific instructions, with 47/48 laboratories returning their results in dual reporting system. Results from the further cycles in 2011-2012 showed an increase in the number of participants and slightly reduced compliance as regards reporting units, indicating necessity for further education on the subject.

Our results reveal a significant improvement in availability and analytical quality and identify EQAS as a valuable tool in harmonization of HbA1c testing in Croatia.

S0 - 7

Postanalytical phase as a modul in EQA – experiences in laboratory haematology

Nazor Aida

Merkur University Hospital, Institute of Clinical Chemistry and Laboratory Medicine, Zagreb, Croatia

Corresponding author: aida.nazor@gmail.com

The Committee for External Quality Assessment of Croatian Society of Medical Biochemists was included in the three projects in the field of laboratory haematology, coordinated by the European Organization for External Quality Assurance Providers in Laboratory Medicine (EQALM). One was in the preanalytical phase regarding the stability of the samples for the preparation of blood films for the morphological analysis and two in the postanalytical phase.

The postanalytical phase projects were conducted internationally including 151 laboratories in 2010 and 318 laboratories in 2011. The goal was to examine the synchronization of evaluation of the haematological results on 4 analytical systems (Sysmex, Advia, Cell-Dyn i ABX) in 12 European countries. The original complete blood count (CBC) of the same clinical case from these haematological analyzers was presented. Results from additional analyses, communicating with medical doctors and various laboratory reports were obtained by asking all participants the same questions.

The obtained results are presented statistically according to the type of analyzer and to the country that has participated. These show the CBC results depend on the type and performance characteristics of the analytic system; management of the analytic system and the degree of personnel education.

There are variations between laboratories in the way they operate and perform standard haematological procedures, particularly regarding analysis and interpretation of the obtained results and taking corrective actions. We have noticed differences which depend upon geographical position of the participant laboratory, and differences that depend upon degree of education and organization of health system in each country.

Symposium lectures

S1 - Can we predict the outcome in critically ill patients?

S1 - 1

Ionized calcium: what to report?

Baird Geoffrey

University of Washington, Laboratory Medicine, Seattle, United States

Corresponding author: gbaird@u.washington.edu

Measuring the calcium concentration in blood is often part of the care of critically ill patients. Many disease states disrupt the complex, multi-organ endocrine system that normally regulates calcium concentration, and assessment of calcium concentration permits one to assess and correct any disturbance and thereby prevent untoward sequelae. While calcium can be measured in several ways - total calcium, albumin-corrected calcium, ionized calcium, and pH-corrected ionized calcium - no method is perfect in all respects. Methods that accurately assess the concentration of the physiologically relevant free cation in solution, so-called "ionized calcium", are more costly and manual, whereas methods that measure the total calcium concentration with or without a protein-binding correction factor are less expensive and highly automated. In choosing which method to use, therefore, one needs to consider the factors that introduce errors in the methods that employ corrections or estimations. The confounders of estimated or corrected values of calcium include blood pH and the concentrations of binding proteins, small molecule chelators, and free fatty acids, and the concentrations of these substances are often abnormal in the blood of critically ill patients. Therefore, the most accurate assessment of the (patho)physiologic calcium concentration in a critically ill patient is one that integrates the effect of the variables present in the patient at the time of measurement. Exceptions to this approach include situations in which preanalytical errors, such as exposure to air, have knowingly altered one or more properties of the sample.

S1 - 2

Glucose regulation in ICU patients; Do's en Dont's

Ligtenberg Jack

University Medical Center Groningen (UMCG), The Netherlands, Emergency Medicine, Groningen, Netherlands

Corresponding author: j.j.m.ligtenberg@umcg.nl

The outcomes of intervention studies implementing intensive insulin therapy aimed at tight glucose control (TGC) are yet not conclusive. Currently, four truly randomized clinical trials evaluating the clinical benefit of TGC have been performed. Out of these four trials, three were negative from a mortality point of view. Obtaining TGC in a real-life ICU setting may result in varying, sometimes disappointing, results. There is concern about an increasing incidence of hypoglycemic episodes. Computerized protocols give the best results and fewer hypoglycemic episodes. Point-of-care blood gas/glucose analyzers present the best trade-off between accuracy and speed of measurement. Closed-loop systems are not yet available for clinical use. Clinicians should take care in selecting both the patient group and target blood glucose level. Although consensus is that frank hyperglycemia (i.e. levels over 8.5–10 mmol/L [150–180 mg/dL]) should not be tolerated, the optimal target is far from clear. A target glucose level of 7–8 mmol/L has been is advised, below which the association between glucose level and adverse outcome subsides. As long as doubts remain about the potential benefits, it is important to perform TGC in a safe way. This can be done with a nurse-driven (computerized) protocol, using arterial blood samples measured on a point-of-care blood gas analyzer. Insulin administration should be continuous. Periodical monitoring of performance and modification of the protocol leads to best results.

S1 - 3

Can lactate-guided therapy improve the outcome in critically ill patients?

Funk Georg-Christian

Otto Wagner Hospital, Department of Respiratory and Critical Care Medicine, Vienna, Austria

Corresponding author: georg-christian.funk@meduniwien.ac.at

Sepsis and septic shock are severe conditions with high mortality requiring intensive care treatment. Therefore sensitive and specific markers of therapeutic success or failure are required. Lactate clearance is the percent change of the plasma lactate concentration during a specified time. Multiple observational studies in patients with various forms of shock showed that lactate clearance < 20% over 2 hours is associated with increased mortality. Moreover two randomized trials in critically ill patients have shown the usefulness of lactate clearance for the management of sepsis and shock. Lactate clearance should be used as an adjunct to the standard measures of goal directed sepsis therapy such as venous oxygen saturation, central venous pressure, urine output and hemoglobin. In summary a lactate clearance > 20% over 2 hours should be targeted in critically ill patients with severe sepsis or septic shock and elevated plasma lactate.

S2 - Kidney biomarkers and formulas

S2 - 1

Estimating glomerular filtration rate in 2012: the creatinine-based equations

Delanaye Pierre

University of Liège, Nephrology-Dialysis-Transplantation, Liège, Belgium

Corresponding author: pierre_delanaye@yahoo.fr

The best overall index of renal function is the glomerular filtration rate (GFR). Since measuring GFR can be cumbersome and costly, estimation of GFR is essential for diagnosis and evaluation of chronic kidney disease, defined as kidney damage or GFR < 60 mL/min/1.73m^2 for ≥ 3 months. Serum creatinine is the classical biomarker used to estimate GFR. Because serum creatinine is also dependent on muscular mass, several authors proposed different creatinine-based equations to estimate GFR. Among these equations, the MDRD and CKD-EPI equations have been shown to be the most accurate. We will describe the advantages but also the limitations, including analytical limitations, of such equations. We will comment the differences of performance between the MDRD versus the CKD-EPI equations. We will eventually underline clinical situations where measuring GFR by a reference method is still required.

S2 - 2

New kidney biomarkers

Kes Petar

University Hospital Centre Zagreb, Department of Internal Medicine, Division of Nephrology, Arterial Hypertension, Dialysis and Transplantation, Zagreb, Croatia

Corresponding author: keszpeter@net.hr

An ideal biomarker of acute kidney injury (AKI) or chronic kidney disease (CKD) should identify the primary location of injury, address the duration of kidney failure, identify the aetiology, stratify risk and estimate prognosis, define the course of the disease and allow the monitoring of response to interventions.

Serum creatinine is a suboptimal marker following injury. In the setting of AKI, the delay between changes in serum creatinine and changes in GFR inhibits the ability to accurately estimate timing and severity of injury. Human neutrophil gelatinase-associated lipocalin (NGAL) seems to be one of the earliest markers in the kidney after ischaemic or nephrotoxic injury may be detected in the blood and urine of humans soon after AKI and de-

layed graft function in kidney transplantation. In AKI, urinary excretion of cystatin C has been shown to predict the requirement for renal replacement therapy earlier than creatinine. Kidney injury molecule-1 (KIM-1) seems to be highly specific for ischaemic AKI and not for pre-renal azotaemia, CKD or contrast-induced nephropathy N-acetyl-β-D-glucosaminidase (NAG) has been shown to function as a marker of kidney injury, reflecting particularly the degree of tubular damage. Interleukin-18 (IL-18) is a pro-inflammatory cytokine detected in the urine after acute ischaemic proximal tubular damage. A single marker may not satisfy the requirement of predicting AKI or CKD progression and mortality. It is more likely that a focused panel of biomarkers will be most rewarding.

S2 - 3

Microalbuminuria: immunoassay and/or HPLC

Wittmann Istvan

University of Pécs, Faculty of Medicine, 2nd Department of Medicine and Nephrological Center, Pécs, Hungary

Corresponding author: istvan.wittmann@aok.pte.hu

Urinary albumin excretion is a good marker of development and progression of kidney, cardiovascular, and probably of some gastrointestinal diseases. The uniform use of this marker in these heterogeneous pathologies is questioned in the last a few years, because excreted albumin and albumin fragments, oxidation, carbonylation and other modifications of the albumin are different in each disease. Many methodologies are used for the determination of albumin in the urine, as e.g. immunoassay, HPLC, LC-MS. Current recommendation prefer immunoassay using polyclonal sera and albumin/creatinine ratio, but identified some clinical needs for standardization. Recently a size-exclusion liquid chromatography method has been developed for the identification of intact, monomer albumin in the urine. This new approach using HPLC have shown that there are albumins in the urine not reacting with the antibodies used in the immunoassay. Thus, in diabetic patients, in the normo- or in the microalbuminuric range a 2-3 times higher albumin excretion was detected by HPLC compared to the immunoassay. This raised a novel hypothesis about the existence of the immuno-unreactive, nonimmunoreactive or immunochemically nonreactive albumin in the urine. The exact nature of this immuno-unreactive albumin is not known and the clinical significance is also matter of debate. This HPLC method is not validated in non-diabetic kidney diseases and seems to be not measuring albumin in inflammatory bowel diseases, since albumin peak consisted mainly of alfa1-acid-glicoprotein and Zn-alfa2-glicoprotein.

S3 - The bleeding patient

S3 - 1

Disseminated intravascular coagulation-clinical approach and lab orientation

Paraskevopoulou Penelope

Konstantopoulio General of Nea Ionia, Hematology Lab, Athens, Greece

Corresponding author: pparaskevopoulou@hotmail.com

Disseminated intravascular coagulation (DIC) is an acquired disorder of hemostasis and is estimated to be present in 1% of hospitalized patients. DIC is a thrombohemorragic disease characterized by the systemic (not local) intravascular activation of coagulation, activation or impairment of fibrinolysis, activation of inflammatory cytokines, complement activation and endothelium damage. These conditions lead to excess thrombin generation, intravascular deposition of fibrin, microvascular thrombosis, consumption of clotting factors, inhibitors and platelets, inadequate removal of fibrin deposition in small vessels, tissue ischemia, necrosis and organ failure with clinical evidence of an excessive bleeding.

The clinical diseases related with DIC are sepsis, severe infections, severe trauma, malignancies, transfusion complications, obstetrical complications (HELLP syndrome, placenta detachment, amniotic fluid embolism) etc.

The diagnosis of DIC is based on clinical and laboratory findings. The main clinical finding is sudden bleeding from multiple sites. Lab findings for DIC are: elevated D-dimers (fibrin degradation products), elevated levels of soluble fibrin monomers (sFM), prolongation of prothrombin time (PT), prolongation of activated partial thromboplastin time (aPTT), low fibrinogen levels, thrombocytopenia and presence of schistocytes on blood smear.

The indicated therapy is the treatment of the underlying disease, otherwise blood products are used to manage the bleeding.

S3 - 2

The use of new thrombelastography (TEG) technologies in critically bleeding patients

Johansson Per

Section for Transfusion Medicine, Capital Region Blood Bank, Rigshospitalet, Copenhagen, Denmark

Corresponding author: rh08061@rh.dk

Introduction: Death due to trauma and massive transfusion is the leading cause of lost life years worldwide, with haemorrhage being responsible for 30-40% of trauma mortality and accounting for almost 50% of the deaths the initial 24h. Furthermore, development of coagulopath is associated with a several-fold increase in morbidity and mortality.

Recent findings: Plasma-based routine coagulation tests (RCoT), like prothrombin time (PT) and activated partial thromboplastin time (APTT), are inappropriate for monitoring coagulopathy and guide therapy in critically ill bleeding patients. Instead viscoelastic haemostatic assays such as Thrombelastography (TEG®) should be used. Clinical studies including more than 5.000 surgical patients have consistently reported on the benefit of using the TEG to identify coagulopathy, predict need for transfusions including massive transfusion and enable goal-directed therapy. New TEG assays such as TEG functional fibrinogen and TEG platelet mapping enables identification of coagulopathies secondary to fibrinogen deficiency and the effect of platelet inhibitors respectively and thereby an even more detailed picture of the individual patients hemostatic competence. Also, RapidTEG enables identification of impaired clot development/ strength within 5 min, which is of particular value in patients with life-threatening bleedings.

Conclusion: Routine use of viscoelastical hemostatic assays is recommended by international guidelines concerning patients with critical bleedings.

S3 - 3

Laboratory assessment of bleeding risk

Alberio Lorenzo

Inselspital, Universitätsspital Bern, Universitätsklinik für Hämatologie und Hämatologisches Zentrallabor, Bern, Switzerland

Corresponding author: lorenzo.alberio@insel.ch

During this lecture I will address: 1) The prevalence and type of bleeding disorders among an unselected patient population scheduled for elective surgery, 2) The predictive values of standard laboratory tests for identifying specific haemostatic defects, 3) The utility of a structured bleeding history for identifying patients requiring a detailed pre-surgical laboratory work-up, and 4) The clinical relevance of laboratory-based and point-of-care-based assays for identifying patients at increased bleeding risk.

S4 - Myeloproliferative neoplasms: diagnosing and molecular monitoring of targeted therapies

S4 - 1

Chronic myeloid leukemia – diagnostics and treatment according to ELN recommendations

Mueller Martin M.

Medizinische Fakultät Mannheim der Universität Heidelberg, Scientific Laboratory, III. Medizinische Klinik, Mannheim, Germany

Corresponding author: Martin.Mueller@medma.uni-heidelberg.de

Chronic myeloid leukemia (CML) has become a model disease since the introduction of the first clinically effective tyrosine kinase inhibitor imatinib. The potent reduction of leukemia load gave rise to the need for more sensitive methods of monitoring. These were realized by the establishment and further development of quantitative polymerase chain reaction techniques. The value of different methods of monitoring (conventional cytogenetics from bone marrow metaphases versus RQ-PCR from peripheral blood samples) will be described according to published data. This presentation will review the current ELN recommendations for monitoring and treatment and outline the most recent publications about new drugs and tighter milestones to be fulfilled in the future of modern CML management. Further the prerequisites of treatment discontinuations will be discussed within the scope of pilot study data.

S4 - 2

Measuring response to tyrosine-kinase inhibitors in chronic myeloid leukemia – molecular monitoring

Zadro Renata

University Hospital Center Zagreb, Department of Laboratory Diagnostics, Zagreb, Croatia

Corresponding author: rzadro@mef.hr

Therapy with tyrosine-kinase inhibitors (TKI) in newly diagnosed chronic myeloid leukemia patient progressively reduces the number of Philadelphia positive cells. As the level of bcr-abl1 fusion transcript correlates well with the number of leukemic cells, molecular monitoring of TKI therapy by quantitative polymerase chain reaction (RQ-PCR) is essential part of patient management. According to European LeukemiaNet (ELN) guidelines, optimal molecular response is defined as major molecular response (MMR = 0.1% bcr-abl1/abl1 according to International Scale - IS)) and complete molecular response (CMR). Molecular monitoring is carried out every 3 months until MMR is achieved and then at least every 6 months to confirm MMR and to reach possible CMR. Molecular monitoring is important to predict therapy response as MMR reduces the risk for disease progression. Earlier achievement of MMR means also higher probability to achieve CMR and stability of response. The importance of CMR detection is that CMR is accomplished in higher percentage with second generation TKI. Major tasks of the EUTOS for CML (European Treatment and Outcome Study for CML, supported by Novartis) within the ELN is to promote the standardization of bcr-abl1 detection via RQ-PCR, as well as definition and standardization of CMR. Active participant in this project is Department of Laboratory Diagnostics, University Hospital Center Zagreb which serves as a reference laboratory for Croatia, Bosnia and Herzegovina, Macedonia, Serbia and Albania for the assignment of a conversion factor in the process of harmonization of quantitative results according to IS.

S4 - 3

Classification and diagnosis of myeloproliferative neoplasms (MPN)

Kušec Rajko

University of Zagreb School of Medicine and Dubrava University Hospital, Department of Hematology and Division of Molecular Diagnostics and Genetics, Zagreb, Croatia

Corresponding author: rkusec@irb.hr

World Health Organization classification for hematologic malignancies of 2008 under the term myeloproliferative neoplasms recognizes chronic myelogenous leukemia Philadelphia-positive, chronic neutrophilic leukemia, polycythemia vera (PV), primary myelofibrosis (PMF), essential thrombocythemia (ET), chronic eosinophilic leukemia NOS, mastocytosis and myeloproliferative neoplasms, unclassifiable. In research of biology of these diseases the discovery of frequent point mutation *V617F* in *JAK2* gene has triggered huge effort in explaining its importance in pathogenesis, but also in diagnosing and beyond in MPNs. This led to detection of other JAK-STAT pathway activating mutations: exon 12 of JAK2 in roughly one third of V617F-negative PV, mutations of the MPL gene in V617F negative ET and PMF. Further, mutations of the CBL (Casitas B-cell lymphoma gene) in PMF, but not in ET or PV were recently found. Mutations of TET2 (TET oncogene family member 2) were described in PV, ET, PMF and in post-PV/post-ET myelofibrosis. The EZH2 was detected in smaller proportion of PMF patients, while CMML was observed with a high frequency of TET2, CBL, and EZH2 mutations. Very soon after new genetic discoveries their clinical evaluation in MPN diagnostic workup has been evaluated and the latest algorithms for application of genetic molecular markers will be presented. The basic view for the rationale use of molecular test in MPN is to start with JAK2V617F mutation and investigate JAK2V617F-negative patients for JAK2 exon 12 or MPL mutations, respectively. In case a MPN cannot be established, analysis for TET2, CBL, and EZH2 mutations may be indicated.

S5 - The inflamed gut

S5 - 1

Microbial sensing and epithelial cell function in IBD

Haller Dirk

Technical University München, ZIEL – Research Center for Nutrition and Food Science, CDD – Center for Diet and Disease, Freising, Germany

Corresponding author: haller@wzw.tum.de

Inflammatory bowel diseases (IBD), comprising the idiopathic pathologies ulcerative colitis and Crohn´s disease, are chronic disorders of the gastrointestinal tract. Genetic susceptibility and environmental factors are crucial parameters in the pathogenesis of IBD. Clinical data and experimental studies using gnotobiotic IBD models revealed that the intestinal microbiota plays a major role in the progression of the chronic intestinal inflammation. In this context, commensal bacteria of the intestinal microbiota can have deleterious or protective effects in the disease-susceptible host. First, we identified the 31.5 kDa metalloprotease gelatinase E (GelE) from *E. faecalis* OG1RF as a putative colitogenic structure. In order to investigate the role of GelE in the development of chronic intestinal inflammation, we monoassociated germfree wild type (Wt) and IL-10-/- mice with *E. faecalis* OG1RF and two isogenic mutant strains TX5264 and TX5266, both lacking GelE expression. Ussing chamber experiments with distal colon segments from non-inflamed IL-10-/- and TNFΔARE/Wt mice revealed the effect of GelE on epithelial barrier function before histological changes have occurred in the tissue. Second, we identified a cell-surface protein of the probiotic VSL#3-derived single strain Lactobacillus paracasei (L.p) mediating anti-inflammatory effects in chronic intestinal inflammation. VSL#3-derived L.p expresses a prtP-encoded lactocepin that selectively degrades IP-10 and other pro-inflammatory chemokines in the diseased tissue environment. In conclusion, we identified two bacterial proteases that displayed in

the context of chronic intestinal inflammation pro- or anti-inflammatory activity.

S5 - 2

Is the disease course predictable through biomarkers in inflammatory bowel disease?

Lakatos Peter Laszlo

Semmelweis University, 1st Department of Medicine, Budapest, Hungary

Corresponding author: kislakpet99@gmail.com

Clinical presentation and disease course of inflammatory bowel diseases (IBD) are heterogeneous and variable over time. During follow patients with Crohn's disease (CD) may eventually one day develop a structuring or a perforating complication and ulcerative colitis (UC) patients, and a significant number will undergo surgery/colectomy. Much emphasis was laid in recent years on the determination of important predictive factors. Laboratory markers have been investigated in IBD for diagnosis, assessment of disease activity, prediction of relapse, risk of complications and monitoring the efficacy of therapy. Markers can be classified as short term (e.g. CRP, ESR) or long term (e.g. serology and genetic) markers. CRP is the most studied and has the best overall accuracy. It correlates well with disease activity and risk for relapses in CD and to lesser extent in UC. More recently, it was shown to be associated with the risk of surgery. However, its use should be individualized, since a proportion of IBD patients fail to mount a CRP response. Other laboratory markers, including ESR, leucocyte and platelet count, have been studied either less extensively. Faecal markers (e.g. calprotectin) seem promising and are more specific in detecting active inflammation in the gut. Recent data suggest that the accuracy of calprotectin is superior in UC compared to CD. Long term markers including serology (e.g. ASCA, pANCA, glycans) or genetic markers(e.g. NOD2/CARD15) may assist the evaluation of disease phenotype in selected cases given that they all were shown to be associated with complicated disease phenotype and risk for surgery, especially in CD. In conclusion, laboratory markers are useful and-together with clinical and endoscopic markers-should be part of the global management of the IBD patients.

S5 - 3

Biological therapy for ulcerative colitis beyond anti-TNF

Lowenberg Mark

Academic Medical Center, Dept. of Gastroenterology and Hepatology, Amsterdam, Netherlands

Corresponding author: m.lowenberg@amc.uva.nl

Ulcerative colitis (UC) is one of the two major types of inflammatory bowel disease, along with Crohn's disease. The medical treatment of UC has received more and more attention from clinicians, researchers and the pharmaceutical industry over the last years. Although the management of UC has improved significantly since the introduction of anti-tumor necrosis factor antibodies, a considerable proportion of patients have a poor response to these agents. Fortunately, elucidating molecular pathways in UC pathogenesis is opening avenues for development of novel therapeutic strategies. Various biologicals and small molecules that selectively target inflammatory mediators have been designed and evaluated in clinical trials demonstrating promising outcomes. Vedolizumab is a monoclonal antibody directed against alpha4beta7 integrin and proved to be effective as induction and maintenance therapy for patients with moderate to severely active UC. Anrukinzumab, an antibody that specifically binds to interleukin-13, is currently undergoing phase II trials. Another promising therapeutic agent is the oral JAK3 inhibitor tofacitinib that is being evaluated in large phase II clinical trials for UC. Finally, the potential therapeutic implications will be discussed of the modification of luminal contents by faecal transplantation.

S6 - Early detection of COPD: biochemical mechanism revisited

S6 - 1

COPD heterogeneicity - clinical phenotypes

Popović-Grle Sanja

Zagreb University Hospital Center, Jordanovac Department for Lung Diseases, Zagreb, Croatia

Corresponding author: sgrle@post.htnet.hr

Chronic obstructive pulmonary disease (COPD) is disease with high morbiditiy and mortality. It is estimated that there are 300 billion COPD patients, double than diabetics, prevalence still increasing. About 65% of patients do not have established diagnosis. Reason for underdiagnosing is probably slowly progression of symptoms, as well as development in eldery. Important reason also is COPD hetegeneicity. COPD is a heterogeneous condition in terms of clinical presentation, underlying pathological mechanism, lung function measurements, systemic manifestations, comorbidities or treatment response.

The oldest hetegeneicity in COPD was recognized as different clinical phenotypes before 50 years. A typical patient with chronic bronchitis is obese person with livide lips, always sleepy, called "blue blotter", while typical person with emphysema is cachaexic, rose lips, visibly searching for more air, called "pink puffer". Today we think that the most important COPD characteristics that define different clinical phenotypes are presence or not of clinical symptoms such as cough and expectoration, with dyspnea, presence or not of frequent exacerbations (more than 2 per year), presence or not of cardiovascular comorbidities, osteoporosis, or depreesion, presence or not of cachaexia or obesitas, presence or not of systemic inflammation, and presence or nor of bronhodilator response in obstructive airways. Hetegeneicity in COPD is big challenge for each clinician, as well as for researchers, trying to find what is common to all COPD patients.

S6 – 2

Biomarkers based on pathophysiology

Abstract not provided.

S6 – 3

Airway epithelial cells alarm the immune system in chronic obstructive pulmonary disease

van Oosterhout Antoon JM.

University Medical Center Groningen, Pathology & Medical Biology, Groningen, Netherlands

Corresponding author: a.j.m.van.oosterhout@umcg.nl

COPD is characterized by chronic neutrophilic airway inflammation triggered by cigarette smoke (CS), leading to bronchitis or emphysema. Little is known about the initial phase of COPD pathogenesis. We showed that CS exposure of airway epithelial cells induces oxidative- and ER-stress responses ultimately leading to cell death. Interestingly, CS inhibits caspase-3 and 7 activities, abrogating apoptotic cell death and promoting necrotic cell death. In contrast to apoptotic cell-death, necrotic cell death leads to release damage-associated molecular patterns (DAMPs) that activate innate immune responses. Indeed, increased DAMPs levels were demonstrated after CS exposure of airway epithelial cells. These DAMPs were shown to induce Myd88-dependent IL-8 production in naïve epithelial cells indicating a role for innate immune receptors.

Furthermore, CS-induced neutrophilic airway inflammation in mice is preceded by epithelial sloughing and of the presence of DAMPs in BAL fluid. The profile of DAMPs and neutrophilic inflammation is genetically determined as shown by comparing different inbred mouse strains.

These early inflammatory responses to CS may be an important initial step to COPD development and may thus offer opportunities for early diagnosis and disease intervention.

S7 - Osteoporosis: investigations, fracture risk assessment and monitoring of treatment. A symposium sponsored by the Asian Pacific Federation of Clinical Biochemistry.

S7 - 1

Diagnostics of osteoporosis and fracture risk assessment

Palicka Vladimir

School of Medicine Hradec Kralove, Charles´ University Prague, Hradec Kralove, Czech Republic

Corresponding author: palicka@lfhk.cuni.cz

Osteoporosis is a systemic, progressive disease of the skeleton, characterized by the decrease of bone mineral content, damaged bone microarchitecture, and increased bone fragility. The ideal diagnostic method should be the measurement of bone fragility, which is the main therapeutic target. Bone fragility cannot be measured in clinical practice and we use surrogate markers.

Clinical examination is of limited use as clinical signs are less expressed and are mostly non-specific.

Common and broadly accepted method for osteoporosis diagnosis is the measurement of Bone Mineral Density (BMD) by Dual-Energy X-Ray Absorptiometry (DXA). BMD is well standardized and results (usually expressed as T-score) correspond to the increased fracture risk. On the other hand, BMD could be very different in different sites of skeleton and more fractures are observed in patients with osteopenia than with osteoporosis.

Bone markers reflect bone remodelling. Increased rate of remodelling is connected to increased fracture risk. Low level of standardization and many influences from out-of-the skeleton decrease the usefulness of bone markers in diagnostics; they are mostly used for monitoring of treatment and patient´s compliance.

Direct assessment of bone microarchitecture by histomorphometry cannot be broadly used in the diagnostics as the procedure is invasive and painful.

New methods like trabecular pattern score (TBS) or HRpQCT provided new insights into the bone structure; the relation with fracture risk is under deep investigation. Individual fracture risk assessment could be estimated by calculation tools - FRAX or FRC.

S7 - 2

Bone turnover markers in the management of osteoporosis

Thomas Devika

SA Pathology and University of Adelaide, Chemical Pathology, Adelaide, Australia

Corresponding author: devika.thomas@health.sa.gov.au

Diagnosis of osteoporosis is based on bone mineral density and in many instances the decision to treat depends on fracture risk. Bone turnover markers have been shown to reflect the rate of bone loss particularly in postmenopausal osteoporosis, as measured by decreasing bone density. However, once diagnosed and treatment is commenced, bone turnover markers may be more useful for monitoring treatment response than bone mineral density due to the dynamic nature of bone markers. Both resorption and formation markers may be useful in monitoring response to therapy and in identifying non compliance.

Markers of bone turnover are proteins released by osteoclasts and osteoblasts during the bone remodelling cycle. With the advent of quantitative laboratory measurements of bone turnover markers, they have become useful in predicting and assessing the rate of bone turnover, although they are affected by a variety of physiological and pathological factors. The pattern of increase or decrease in bone turnover makers is not disease specific, therefore they are often not useful in diagnoses. However, the change in the pattern

may be useful in fracture prediction, monitoring treatment response and in identification of non compliance or non response. Various clinical guidelines now incorporate bone turnover markers in monitoring therapy, and the role of bone mineral density in early monitoring of therapy has been questioned. With refined laboratory techniques and methods, more accurate and precise bone turnover marker measurements, they may be incorporated into various algorithms in diagnosis, decision to treat and monitoring disease progression.

S7 - 3

Vitamin D status and osteoporosis: evidence for a role in hip and vertebral fractures

Morris Howard

University of South Australia and SA Pathology, Adelaide, South Australia, School of Pharmacy and Medical Sciences and Chemical Pathology, Adelaide, Australia

Corresponding author: howard.morris@health.sa.gov.au

Current data demonstrate that vitamin D deficiency contributes to the aetiology of at least two metabolic bone diseases, osteomalacia and osteoporosis. Osteomalacia, or rickets in children, is the index disease of vitamin D deficiency and arises from a delay in mineralization. It can be resolved by normalising plasma calcium and phosphate homeostasis even if the vitamin D status remains depleted. The well characterised endocrine pathway of vitamin D metabolism and its activities are solely responsible for vitamin D regulating plasma calcium and phosphate homeostasis and therefore for protecting against osteomalacia.

In contrast, a large body of clinical data supports the concept that an adequate vitamin D status protects bone health by improving bone mineral density and reducing the risk of fracture. The risk of hip fracture is markedly increased at levels of serum 25-hydroxyvitamin D below 50 nmol/L. Meta-regression analyses of data from RCTs of vitamin D supplementation suggest that serum 25-hydroxyvitamin D levels above 75 nmol/L are required to achieve significant fracture reduction. A plausible mechanism for various activities arising from serum 25-hydroxyvitamin D levels greater than 50 nmol/L is provided by studies demonstrating metabolism to 1,25-dihydroxyvitamin D by each of the major bone cells to activate VDR and modulate gene expression to reduce osteoblast proliferation and stimulate osteoblast and osteoclast maturation and mineralisation.

S8 - Laboratory genetics - the critical importance of genotype and phenotype, and their correct interpretation

S8 - 1

MODY - which genes to test?

Ellard Sian

University of Exeter Medical School, Institute of Biomedical and Clinical Science, Exeter, United Kingdom

Corresponding author: sian.ellard@nhs.net

Maturity-onset diabetes of the young (MODY) is a monogenic subtype of diabetes that can be caused by mutations in multiple genes. The most common forms are caused by mutations affecting the glycolytic enzyme glucokinase (GCK gene) or the transcription factor HNF-1 alpha (HNF1A gene) and cause either mild fasting hyperglycaemia throughout life (GCK) or progressive diabetes diagnosed in adolescence/adulthood with risk of diabetic complications (HNF1A). Treatment is determined by the genetic subtype since patients with GCK mutations rarely need pharmacological therapy whereas those with HNF1A mutations are sensitive to sulphonylurea tablets. However, most patients (~90%) are misdiagnosed as having type 1 or type 2 diabetes and access to genetic testing varies 10 fold across the UK.

Selection of patients for MODY genetic testing can be facilitated by non-genetic tests. These include

pancreatic autoantibody and urinary C-peptide tests to identify those more likely to have type 1 diabetes and a web-based MODY predictor tool that gives a likelihood estimate that a patient has MODY.

S8 - 2

The challenges of molecular genetic testing in inherited cardiomyopathy

Thomson Kate

Oxford University Hospitals NHS Trust, The Churchill Hospital, Oxford Medical Genetics Laboratories, Oxford, United Kingdom

Corresponding author: kate.thomson@ouh.nhs.uk

Hypertrophic cardiomyopathy (HCM) is the most common inherited cardiac disorder with an estimated frequency of 1/500. It is a clinically important condition being the most frequent cause of sudden cardiac death (SCD) in young people and trained competitive athletes. HCM is clinically heterogeneous exhibiting variable age of onset, penetrance and expressivity. It is also genetically heterogeneous with over 13 genes reported to be associated. Although generally considered to be an autosomal dominant trait, other more complex patterns of inheritance are becoming apparent. The potential benefits of genetic testing in this condition are widely recognised, however, pathogenic mutations remain elusive in approximately 50% of patients. The challenges of genetic testing in this clinically and genetically heterogeneous condition will be discussed and future analysis strategies explored.

S8 - 3

Familial hypercholesterolaemia: genetic testing helps, but should be used with care

Abstract not provided.

S9 - The poisoned patient

S9 - 1

Modern methods in clinical and analytical toxicology

Grobosch Thomas

Labor Berlin - Charité Vivantes GmbH, Laboratoriumsmedizin & Toxikologie, Berlin, Germany

Corresponding author: thomas.grobosch@laborberlin.com

Background: Acute or chronic poisoning is an emergency situation require immediate, unequivocal and reliable detection and quantification of xenobiotics in blood or plasma/serum in order to establish the correct treatment for the patient. For the detection of unknown substances in such complex biological matrices, only methods providing high specificity and sensitivity are appropriate. Another important aspect is the continuous availability of the complete analytical instrumentation. Basically, toxicological analysis always has to be a compromise between demand of time needed for the procedure and the accuracy of the analytical results.

Methods: Concepts and procedures using e.g. immunoassays/photometric tests, headspace gas chromatography coupled to e.g. flame ionization detector (HS-GC-FID), liquid chromatography coupled with diode-array-detector (HPLC-DAD) or time-of-fight (TOF) analyzer are presented and their perspectives of their future are discussed.

Results: When only a single drug or category has to be monitored, immunoassays/photometry can be used for preliminary screening in order to save time and costs (e. g. THC or lithium). For the systematic toxicological analysis of unknown substances, only the combination of chromatographic systems coupled to a diode-array-detector (identified or classified by their UV-spectra) or a TOF mass analyzer (identified by their accurate mass) will be able to cover the majority of toxicological relevant substances in a reasonable short time. For the quantification of volatile compounds, a HS-GC is absolutely indispensable.

Conclusions: Certain immunoassays/photometric tests, HS-GC-FID, HPLC-DAD, and time-of-fight (TOF) techniques are indispensable modern tools to cover a broad range of xenobiotics and the rising challenges in clinical and analytical toxicology.

S9 - 2

The poisoned patient: quantification in emergency toxicology?

Uges Donald R. A.

University Medical Center Groningen, Hospital and Clinical Pharmacy / Laboratory for Clinical and Forensic Toxicology and Drug Analyses, Groningen, Netherlands

Corresponding author: j.a.tuinstra@umcg.nl

Toxicology is the knowledge of "too much". In other words, toxicology is quantitative science. However, in some clinical toxicology cases, just qualitative analysis in combination with patient's clinical manifestation (toxicodromes) and the available information could be sufficient. To interpret the analytical results, at least one specialist involved should have experiences in pharmacokinetics and toxicokinetics, i.e. an emergency physician, a toxicologist, a hospital pharmacist or a biochemist. Pharmacokinetics is dealing with the time interval of the blood concentration with respect to time of drug intake. Toxicokinetics involves drug exposure and toxicity along with challenges of saturated kinetic parameters and organ failure. Intrinsic low toxic substances can become highly toxic with saturated protein binding. Toxicity can be dependent on at least the dose, concentration, co-medication and patient's clinical condition. The choice and duration of treatment and the prognosis is often concentration dependent. It has to be taken into account that reliable information of the time and route of administration is essential for a good toxicological treatment advice. Nowadays, commonly used analytical methods in clinical toxicology are immunoassays, LC-MS/MS and GC. They all provide both qualitative and quantitative results. In daily practice, therapeutic drug monitoring and clinical toxicology are merging, especially if the cause of poisoning is iatrogenic. Both a too low and a too high dose of an antiepileptic drug can cause seizures.

In conclusion: quantitative analysis can be very useful in clinical toxicology if the right information is provided along with a proper interpretation. (www.bioanalysis.umcg.nl).

S9 - 3

The health risk of recreational drugs and novel psychoactive substances

Wood David Michael

Guy's and St Thomas' NHS Foundation Trust, Clinical Toxicology, London, United Kingdom

Corresponding author: david.wood@gstt.nhs.uk

The use of recreational drugs is common, particularly amongst young people and those who frequent the night-time economy (e.g. bars, raves, discotheques). Recently that has been increasing use and availability of novel psychoactive substances (also known as "legal highs"). These substances include the cathinones (e.g. mephedrone), the piperazines (e.g. 1-benzylpiperazine), the synthetic cannabinoid receptor agonists, the pipradrol-related drugs (e.g. D2PM) and ketamine analogues (e.g. methoxetamine). There is little or no information available on the acute health effects (toxicity) of novel psychoactive substances.

Using a process known as "data triangulation", it is possible to bring together information from a range of different sources to develop an understanding of the overall pattern of acute toxicity for a novel psychoactive substance(s). This reduces the limitations of any one data source, the main one is that typically they is no analytical confirmation of the substance(s) used. These data sources include: Internet discussion fora, where individuals post information on their own personal experiences; case reports/series; poisons centre data series; animal models and human studies.

Classical recreational drugs can clinically be divided into three broad categories based on clinical effects seen with acute toxicity: hallucinogenics (e.g.

LSD, ketamine), depressants (e.g. opioids, gamma-hydroxybutyrate (GHB)), and stimulants (e.g. cocaine, amphetamine, MDMA). Using data triangulation it is possible to conclude that the pattern of acute toxicity of many novel psychoactive substances can similarly be divided: the synthetic cannabinoid receptor agonists and methoxetamine have hallucinogenic-like acute toxicity and the cathinones, piperazines and pipradrol-related drugs have a stimulant-like acute toxicity.

S10 - Education of specialists in laboratory medicine in Europe

S10 - 1

EU perspective in medical specialization

Maillet Bernard

UEMS, Former Secretary-General, Brussels, Belgium

Corresponding author: bernie.mail@skynet.be

In Laboratory Medicine, the big challenge is to harmonise the specialty and the practice in Europe as there are so many different ways in which laboratory medicine is rained and practiced all over Europe.

By proposing a harmonized training program together with the European Federation of Laboratory Medicine in a polyvalent manner, and start to implement this program in an increasing number of EU Member States, the process of harmonization will go hopefully softly and surely forward.

This polyvalency in the training does not exclude that individual practitioners could not have their own field of interest and build by doing so a "Particular Qualficiation" in certain domain(s).

Bearing in mind the revision of the European Directive on the Recognition of Qualifications which is expected to be finalized at the end of 2012, a possibility appears to work on the change of the denomination of the specialty in the Addendum of the Directive.

This will also enable the National Medical Associations and the National Professional Organizations to implement this change at national level and introduce the denomination as well as the common training program as proposed by the UEMS Section of Laboratory Medicine and the EFLM as the national requirement to become specialist in Laboratory Medicine in the different EU Member States.

To be successful, the good collaboration between the different stakeholders in the field is of the utmost importance and this concerns both the European as well as the National level.

S10 - 2

Education and training activities in EFLM

Zima Tomas

Institute of Medical Biochemistry and Laboratory Medicine, First Faculty of Medicine, Charles University, Prague, Czech Republic, Prague , Czech Republic

Corresponding author: zimatom@cesnet.cz

The education and training is one of the important activities of EFLM in different areas. Main goals are to assist in knowledge transfer in all areas of profession, to help implement new diagnostic strategies, to produce and assure the quality of EFLM educational materials, support and organized meeting and conferences, to establish the distance learning systems (e-seminars), to coordinate a prepare postgraduate continuous education credits system for our profession. There are many scientific events with strong educational activity as traditional EFLM Postgraduate Course, held in Dubrovnik, Croatia, EFLM Symposium for Balkan Region, Belgrade, Serbia, EFLM -Becton Dickinson preanalytical conference, cooperation with BioRad on conference with topic - Quality in Laboratory Medicine and Accreditation. The main congress in Educational activity is European Joint Congress of EFLM and UEMS in Lisbon and Dubrovnik, now. New attractive instrument is EFLM e-Seminar Series which started in 2010 and for 2012 is prepare 6 seminars. The key topic is harmonization and compensation the differences in specialization education of our profession Specialist in laboratory medicine. The main activity was the education con-

ference held in Prague in March 2012 which should find the consensus in pregraduate, postgraduate and specialization education in Europe in our profession.

S10 - 3

Evaluation/revision of the European Directive on professional qualifications

Zerah Simone

Centre AMP, clinique de la DHUYS, Laboratoire ZTP, Bagnolet, France

Corresponding author: simone.zerah@gmail.com

Background: The first Directive (2005/36/EC) on Recognition of Professional Qualifications had to be transposed by all Member States by 2007. This document is under revision.

Methods: We will give a quick overview of the history to enable understanding of where we are now, after active participation in the numerous meetings and questionnaires, and stress the importance for the future of our profession. We will present the two methods for recognizing qualifications, the automatic system for 7 "sectoral professions" and the "general system". We will report the propositions of the "green paper" and the subsequent draft of the revised directive, published in December 2011, proposed to the European Parliament by the European Commission.

We will explain our strong involvement, and our propositions on the main points: Professional cards, "Common training principles" replacing the common-platforms, back to harmonization instead of identifying differences for compensation measures, CPD, Partial access, rules on language skills.

Results and conclusion: The Deadline for amendments is 15 October. The European professional associations should be well placed to take the lead in devising harmonization frameworks: our aim is the excellence of our profession in Europe. The new Directive is due to be published by the end of 2012.The European Parliament has officially made it a priority. The Member of the Parliament in charge is Bernadette Vergniaud.

S10 - 4

Can we harmonize laboratory medicine in Europe?

Oosterhuis Wytze

Atrium Medical Centre, Clinical Chemistry, Heerlen, Netherlands

Corresponding author: w.oosterhuis@atriummc.nl

The EFLM and UEMS Section of Laboratory Medicine/Medical Biopathology in 2009 have decided to join forces on several projects. One is a survey, with the aim to make a description of the current state of organizations, practices and responsibilities of laboratory professionals within the European Union. Both the EFLM (EC4 curriculum) and UEMS ("Blue Book") work at harmonizing the training of professionals with medical or scientific background. This is closely related to the free movement of people, a major goal of European integration.

In March 2010 a questionnaire has been sent to representatives of both organisations. Delegates of EU countries were asked to answer questions related to the following subjects: the number of professionals (MD, PhD and other academically trained), content of the laboratory specialty, professional organizations responsible for training, official acknowledgment of training and specialties, length of training, relation of scientific organizations with UEMS and EFLM and accreditation of laboratories. The results will be presented, and show a very diverse situation across Europe in many aspects: the relative number of specialists in Clinical Chemistry, the ratio of specialists with a medical and scientific background, official acknowledgement of the Clinical Chemistry specialties, content of the specialty and responsibilities. In some countries the field of Clinical Chemistry is divided in monospecialties, on other countries there is a broader general or "polyvalent" laboratory specialty. A better understanding of the organization and practice of laboratory

medicine will be of help in the harmonization of Clinical Chemistry across Europe.

S11 - Stroke biomarkers

S11 - 1

The role of blood biomarkers in acute ischemic stoke

Katan Mira

Columbia University, Department of Neurology , Divison of Stroke, New York, United States

Corresponding author: mk3270@columbia.edu

Blood biomarkers have begun to play an increasingly important role in the triage and management decisions in several acute medical diseases.

In cardiovascular diseases, some biomarkers are widely accepted and have been successfully implemented into clinical routine, including troponin for the diagnosis of myocardial infarction and BNP to guide diuretic therapy in heart failure.

In acute ischemic stroke several biomarkers have been identified as potential candidates and some have been investigated in clinical settings. However, the utility of a biomarker is defined by its ability to improve clinical decision-making and add timely information beyond that readily available from clinical examination and routine imaging. This aim has not been completely achieved yet for any biomarkers in acute ischemic stroke.

Unique challenges exist in the identification of clinically useful blood biomarkers in ischemic stroke. Stroke is a heterogeneous disease not only etiologically but also in terms of affected cell types. Depending on the clinical question blood biomarkers should reflect distinct pathological processes within the brain and systemically. The blood brain barrier, which separates the brain from the systemic circulation, is an additional concern in this context.

Despite the inherent difficulties to identify useful blood biomarkers for diagnosis or prognosis in ischemic stroke promising data is available and further studies are ongoing. If well-designed studies confirm the incremental value of diagnostic and prognostic blood biomarkers over existing diagnostic and prognostic tools the advantageous opportunity to use these biomarkers to individualize and optimize patient care will arise.

S11 - 2

The significance of uric acid levels in stroke patients treated with thrombolysis

Amaro Sergio

Hospital Clínic, IDIBAPS, University of Barcelona, Functional Unit of Cerebrovascular Diseases, Barcelona, Spain

Corresponding author: samaro@clinic.ub.es

Stroke is a devastating disease with an imperative need for more effective therapies. The thrombolytic molecule rTPA - recombinant tissue plasminogen activator - is the only licensed drug for acute stroke, but its efficacy is limited. The coadministration of neuroprotective drugs could augment the value of thrombolytic therapy, but the evidence in support of this approach is scarce. Uric acid (UA) is the major endogenous antioxidant in blood, and its concentration is almost 10-fold higher than other antioxidants. In acute stroke patients, there is a rapid consumption of UA after stroke and higher UA levels at stroke admission have been associated with less infarction growth at follow-up. Moreover, increased UA serum levels have been associated with better outcome in patients treated or not with reperfusion therapies. In patients receiving thrombolytic therapy, lower UA levels at stroke onset have been associated with a greater incidence of malignant middle cerebral artery infarctions and/or hemorrhagic transformation of the infarction. In experimental ischemia, the exogenous administration of UA has shown neuroprotective properties. Indeed, the coadministration of UA and rTPA has shown to provide synergistic neuroprotection in experimental thromboembolic models and to lessen several biomarkers of oxidative stress in patients with acute stroke. Nowadays, the clinical efficacy of uric acid in acute ischemic stroke

is under investigation in an ongoing phase IIb/3, randomized, placebo-controlled trial.

S11 - 3

Are admission serum lipids predictive of the stroke outcome?

Pikija Slaven

University Clinical Center Maribor, Neurology Department, Maribor, Slovenia

Corresponding author: spikija@gmail.com

Various functions in living organisms are mediated and encompassed with lipid molecules. They are fundamental components of cell barriers thus enabling essential prerequisite for life – compartmentalization. Beside space limiting function, they also serve as messengers in multicellular organisms. Intrinsic properties of high-energy reduced chemical bonds between carbon atoms are recognized by living cell as perfect way to store energy for later use. In equilibrium lipids serve organism well. However, certain pathological states tend to move lipid physiology in unwanted direction. It is well established fact that serum lipid perturbations in long term, along with permissive inflammation state, carries risk for atherosclerosis and all subsequent adverse effects of later. Those facts are best established for coronary artery disease and heightened level of serum lipid – cholesterol. Some favorable effects on stroke volume in patients with heightened levels of triglycerides as well as linkage between low level of cholesterol with greater risk for intracerebral hemorrhage are reported. One hypothesis argues that intrinsic properties of lipid perturbations leads to favorable state of accessible alternative brain food – blood ketones that is known to have multiple neuroprotective effects. Other hypothesis tries to link effects of cholesterol on platelet aggregation and other to effect of TG to ameliorate fatty-acid induced lipotoxicity. Still some have found that high cholesterol can increase gamma-glutamytranferase which can be neuroprotective. It may be as well that intracranial vascular endotel reacts differently to lipid values, specifically when stroke happens, despite gross similarity between atherosclerotic plaques between extracranial and intracranial lesions.

S12 - Diagnosing and monitoring of DM with POCT instruments

S12 - 1

Diagnosing and monitoring diabetes mellitus with POC instruments

Sandberg Svere

Norwegian Quality Improvement of Primary Care Laboratory, University of Bergen, Bergen, Norway

Corresponding author: sverre.sandberg@isf.uib.no

POC instruments for glucose and HbA1c have been used for a long time to monitor diabetes mellitus. In a monitoring situation it is important that the instruments give reproducible results, and therefore that the patients visit the same facility each time. Because of the varying trueness of glucometers, these instruments have – in most countries – not been recommended for diagnosing diabetes mellitus although some GPs and Pharmacists use POC instruments for case finding and, in case of a "positive" result, forward samples to a larger laboratory for further examination. The same has been the situation with u-albumin where case finding for microalbuminuria has been performed, but samples have been sent to larger laboratories for confirmation. Diabetes mellitus can now be diagnosed using HBA1c and an important question is: Can this be done with POC instruments in primary health care? We suggest a model for quality assurance, similar in hospital laboratories and in laboratories using POC instruments. In this model both the trueness and the precision will be registered by an External Quality control Scheme (EQAS), with an allowable deviation in the EQAS of 7 % and where the intralaboratory (within one lot) should be < 2% for the participant to use A1c as a diagnostic criterion. We have established a system to advice and

educate the participants using POC instruments on how they can improve and thereby use the POC instrument for HbA1c to diagnose diabetes mellitus.

S12 - 2

The role of POC instruments for diagnosing and monitoring u-albumin in persons with diabetes mellitus

Gillery Philippe

University Hospital of Reims, Laboratory of Pediatric Biology and Research, Reims, France

Corresponding author: pgillery@chu-reims.fr

Urine albumin (u-albumin, "microalbuminuria" is discouraged) is a marker of kidney damage and is considered a risk factor for renal disease progression and cardiovascular events in diabetic patients. Its accurate measurement is a necessary prerequisite for the correct use of results in global patient care.

Whereas u-albumin may be measured by various immunoassays in clinical laboratories, POCT instruments have been introduced, that measure urine albumin and creatinine, and calculate albumin/creatinine ratio (ACR). ACR is generally accepted as a valid approach to overcome limitations related to urine collection.

Two main questions must be addressed: (i) Are POCT methods for u-albumin as reliable as central laboratory methods? (ii) What is the added value of u-albumin POCT in patient care?

Recent studies have shown that POCT analyzers exhibited analytical performances allowing their use in clinical situations. However, reference values, especially for ACR, may vary between the different devices, and must be taken into account for clinical interpretation. Thus, POCT evaluation of u-albumin may be useful for diabetologists or general practitioners to identify diabetic patients at risk for cardiovascular complications and progression of kidney disease. Although there are still few studies about the relative benefits of POCT *vs*. laboratory assays for u-albumin evaluation, successful approaches have been reported, especially in Australia for community risk assessment, but also for patient management, in large-scale programs dedicated to remote population.

Like HbA1c, u-albumin may be assessed by POCT methods and may represent an interesting alternative in addition to conventional laboratory methods in particular clinical situations.

S12 - 3

Glucose self monitoring by POCT systems with emphasis on the Italian recommendations

Mosca Andrea

Università degli Studi di Milano, Dip. di Fisiopatologia medico-chirurgica e dei Trapianti, Milano, Italy

Corresponding author: andrea.mosca@unimi.it

The term of self-monitoring (SMBG) refers not only to the measurement of blood glucose by patients, usually at home, but also to the interpretation of the results and to some actions to be undertaken in order to improve the glycemic control. Therefore, SMBG is part of an educational trial to be undertaken in collaboration with health care providers. By looking to the evidences, SMBG is essential for type 1 diabetic patients and for type 2 diabetic patients, insulin treated (levels of evidence II, strength A and B, respectively). On the other hand, non continuous SMBG in type 2 diabetic patients under oral hypoglycemic drugs or simply under controlled diet is useful only if an appropriate education is provided, together with the indication of actions to be undertaken to change the therapy on the basis of the SMBG results (strength II, force B). With regards to the choice of the glucometer, a consensus document recently developed by an Italian task force will be illustrated in its major points. Essentially, with regard to the analytical characteristics, only plasma-calibrated POCT have to be used, with compliance to the ISO 15197/2003. Monitoring of the imprecision (minimum desirable CV ≤ 5 %) and bias can be performed by adequate internal quality control and EQAS procedures, in collaboration with laboratory professionals.

S13 - Pre-pre and post-post analytical aspects: communication between laboratory and clinicians

S13 - 1

Improving pre-analytical, analytical and post-analytical phase by EQAS – examples

Aarsand Aasne Karine

Haukeland University Hospital, Laboratory of Clinical Biochemistry, Bergen, Norway

Corresponding author: aasne.aarsand@helse.bergen.no

Even though most laboratory errors are thought to occur in the pre-analytical and post-analytical phases, EQA schemes typically focus mostly on the analytical phase and testing of the non-analytical phases are only rarely performed. NOKLUS, in cooperation with EQALM, runs a web-based post-analytical EQA scheme for automated haematology instruments including more than 400 laboratories in 14 European countries. Participants, mostly medical laboratory technologists responsible for the instruments, interpret cell counts and plots from five-part differential automated haematology instruments in light of an accompanying case history. Results from several distributions show that there are differences between participants and also between different countries regarding how they work, the standard operating procedures used for interpretation of results and plots and the corrective actions taken. To assess all aspects of the laboratory diagnostic process is even more important for rare metabolic disorders, considering the central role played by the laboratory in establishing these diagnoses. The European Porphyria Network (EPNET) runs a clinical case-based EQA scheme covering pre-analytical, analytical and post-analytical aspects for all biochemical analytes relevant for diagnosing the porphyrias. The participants, at present 33 porphyria specialist laboratories throughout Europe, apply diverse diagnostic strategies and large variations in analytical performance are also typically evident. However, most laboratories provide appropriate interpretations and correct diagnoses. Improvements in applied diagnostic strategies and analytical performance have been observed during the time the EPNET EQA scheme has been running, underlining the importance of offering EQA schemes that test the complete laboratory process for diseases requiring complex diagnostic testing.

S13 - 2

Important quality indicators for the pre-analytical phase

Simundic Ana-Maria

Clinical Hospital Center Sestre Milosrdnice, Clinical Institute of Chemistry , Zagreb, Croatia

Corresponding author: am.simundic@gmail.com

Quality of a service or a product is a measure of the satisfaction of its consumers or customers. Quality of a healthcare service may thus be defined as a measure of its impact or effect on the patient care. Quality indicators are tools aimed to measure the quality of a health care service. They need to be evidence based, available, accessible and measurable, objective and relevant to the particular system. They also must be able to indicate the potential for improvement, within the continuous improvement cycle. Numerous organizations have attempted to identify a set of quality indicators and quality specifications to be used universally by any laboratory worldwide, regardless of its size, setting, type and geographical location. However, up to today there is still no universal agreement on the core set of quality indicators for preanalytical phase. Until such agreement has been reached, each laboratory needs to define its own indicators and way to implement it into its quality system. When implementing quality indicators, there are many issues to be addressed, such as: valid and reliable definition of the indicator, its relevance to the service, accessibility, availability, reporting frequency, available corrective measures, acceptance limits and some other. Quality indicators in the preanalytical phase of the laboratory testing relate to the test ordering, patient identification, specimen collection, transport and handling. Currently, data from different laboratories are difficult to compare and standardization and harmonization in that respect is of utmost importance.

S13 - 3

Standardization and harmonization of quality indicators in the pre- and postanalytical phase

Plebani Mario

University-Hospital, Department of Laboratory Medicine, Padova, Italy

Corresponding author: mario.plebani@unipd.it

Quality in laboratory medicine should be defined as the guarantee that every step in the total testing process (TTP) is correctly performed. Whilst current quality indicators in laboratory medicine tend to focus on the performance and efficiency of analytical processes, recent evidence suggests that most errors in the TTP fall outside the analytical phase. The current lack of attention to extra laboratory factors is thus in stark contrast with the body of evidence pointing to the multitude of errors that continue to occur in the pre-analytical phase.

As a result, in 2008, the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) launched a working group named "Laboratory errors and patient safety" with a goal to identify and evaluate valuable QIs and related quality specifications in order to address all the stages of the TTP. The prerequisites for selected QIs were: a) relevance and applicability to a wide range of clinical laboratories; b) scientific soundness, with a focus on areas of great importance for quality in laboratory medicine; c) feasibility, both regarding the data availability and the definition of thresholds for acceptable performance; d) timeliness and possible utilisation as a measure of laboratory improvement. 56 QIs have been identified, of which 34 in the pre-analytical, 7 in the intra- and 15 in the post-analytical phase. The aims of, and steps taken in, the IFCC-WG program will be described and preliminary results discussed for better addressing future steps of the project particularly as regards pre- and post-analytical indicators.

S14 - Obesity: from genetic loci to early indicators

S14 – 1

Genetic loci and body mass index

Abstract not provided.

S14 - 2

The obese adolescent: conclusions of the HELENA study

Molnár Denes

University of Pécs, Pediatrics, Pécs, Hungary

Corresponding author: denes.molnar@aok.pte.hu

The HELENA Project was a comprehensive three-year research programme, spanning 10 countries, using a common methodology designed to assess the nutritional status and behavior, as well as the fitness and physical activity patterns, of more than 3000 adolescents aged 12.5 to 17.5 years.

The basic objective of the HELENA cross-sectional multicentre study was to obtain reliable and comparable data on dietary intake, food preferences, anthropometry, serum indicators of lipid and glucose metabolism, vitamin and mineral status, physical activity, fitness and genetic markers.

The aim of the lecture is to present a bunch of the most important findings of the HELENA Study in the light of other, recent international results.

The prevalence of BMI (kg/m^2) categories in European adolescents (N = 3528, male = 1683) was the following: male: BMI < 18.5 = 5.1%; 18.5 ≤ BMI < 23 = 51.7%; 23 ≤ BMI < 25 = 16.3%; 25 ≤ BMI < 27 = 10.9%; 27 ≤ BMI < 30 = 8.6%; 30 ≤ BMI < 35 = 6.0%; 35 ≤ BMI = 1.4%; females: BMI < 18.5 = 6.6%; 18.5 ≤ BMI < 23 = 54.5%; 23 ≤ BMI < 25 = 18.5%; 25 ≤ BMI < 27 = 10.0%; 27 ≤ BMI < 30 = 6.3%; 30 ≤ BMI < 35 = 3.5%; 35 ≤ BMI = 0.6%.

The prevalence of overweight and obesity showed a north to south gradient.

42% of normal weight and only 31.5% of obese adolescents spent 60 minutes/day in moderate-to-vigorous physical activity as measured by accelerometer. Adolescents spent most of the registered time in sedentary behaviors (9 hours/day, or 71% of the registered time).

In the HELENA sample 1.1% of children had metabolic syndrome according to the criteria of the International Diabetes Federation.

The results of the HELENA Study beside their scientific merit help authorities to launch preventive measures on a European level.

S14 - 3

The value of obesity research

Oppert Jean Michel

University Pierre et Marie Curie, University Pierre et Marie Curie, Paris, France

Corresponding author: jean-michel.oppert@psl.aphp.fr

Obesity is the most prevalent metabolic disease worldwide. Despite significant recent research investment, obesity prevalence rates continue to rise throughout most countries of the world, not least in Europe. In recent decades, research has helped improve our understanding of the complex effects of increased fat tissue on the human body and has enabled us to develop better evidence based guidelines to prevent, manage and treat obesity, including better bariatric surgery procedures to treat extreme obesity. A major challenge is to increase the translation of this research into better prevention and treatment strategies. In response to this situation, the European Association for the Study of Obesity (EASO) has now completed a two year consultation process, culminating in a meeting of Europe's leading researchers and main stakeholders in the field with the aim of developing a clear research strategy, with consensus on what the key areas for European obesity research should be, the expected impact of this research and how we can achieve success in these areas in terms of novel approaches. EASO recommends that a first priority is to 'Improve obesity diagnosis (beyond BMI) to categorize individuals for appropriate prevention and management, including critical periods of life'. For all recommended research topics, a major focus is on transdisciplinary approach (integrating social sciences and humanities), societal impact and innovation/economic growth. The key to advance obesity prevention and treatment in Europe now require a political commitment on both member state and EU level.

S15 - New trends in laboratory medicine

S15 – 1

Next generation sequencing for clinical diagnostics

Abstract not provided.

S15 – 2

Population screening for genetic disorders in the 21st century

Abstract not provided.

S15 - 3

Towards fast microbiology

Vila Jordi

Hospital Clinic, Clinical Microbiology, Barcelona, Spain

Corresponding author: jvila@ub.edu

In the last decade a plethora of molecular biology tools have been applied for the diagnosis of infectious diseases. These techniques can provide information from clinical samples within only a few hours. Several multiplex real time PCRs have been applied to detect microorganisms causing sepsis, respiratory

tract infections, sexually transmitted infections, including the detection of virus and bacteria in the two former cases. Finally, multiplex real time PCR panels to detect bacteria, virus and protozoa causing gastroenteritis has also been developed. In this sense specific PCR for individual microorganisms have also contributed to the more rapid diagnosis of infectious disease. When a culture is negative and an infectious diseases is suspected, broad PCR based on amplification and sequencing of the 16S rRNA gene (for bacteria) or the ITS region (for fungi) can be carried out. An important breakthrough in the rapid diagnosis of infectious diseases has been the incorporation of MALDI-ToF mass spectrometry not only to rapidly identify bacteria and fungi but also to detect mechanisms of resistance associated with the degradation of the antibiotic, for instance, detection of beta-lactamases. In addition, ESI-ToF mass spectrometry is also used to identify whatever microorganism is found in the clinical sample by using PCR amplification of specific gene(s) and ESI-ToF to detect the exact size of the amplicon and the number of A, T, C and G. Based on a specific algorithm the microorganism is thereafter determined. Therefore, we are witnessing profound changes in the way that microbiological diagnoses are performed.

PC1 - Pharmacogenetics in predicting anti-cancer therapy response

PC1 - 1

Pharmacogenetics in predicting anticancer therapy response: pro

van Schaik Ron

Erasmus MC, Dept. Clinical Chemistry, Rotterdam, Netherlands

Corresponding author: r.vanschaik@erasmusmc.nl

Prediction of adverse drug effects, or on effectivity of therapy seems most promising in Oncology. In this field, toxicity is often dose limiting. Whereas undertreatment will have fatal consequences. Most anticancer drugs, however, are metabolized by CYP3A4, for which no clinical relevant genetic polymorphisms have been described until recently. In contrast, the anti-estrogen tamoxifen, which is used as adjuvant therapy for breast cancer, needs activation to endoxifen, a process which is heavily depending on the genetically polymorphic enzyme CYP2D6. Approximately 5-10% of the Caucasian population is a poor metabolizer for CYP2D6, with almost no CYP2D6 activity due to inheritance of two inactive alleles. An additional 30% is intermediate metabolizer, with reduced CYP2D6 activity. Based on the theoretical involvement of CYP2D6 in the activation of tamoxifen to endoxifen, one would expect lower endoxifen plasma-concentrations in the circulation of CYP2D6 poor metabolizer breast cancer patients and a lower survival. The correlation of CYP2D6 deficient alleles with lower endoxifen concentrations was indeed confirmed. In addition, several studies showed indeed a lower survival for carriers of one or two defective CYP2D6 alleles. From these facts, it is clear that CYP2D6 genotyping for tamoxifen treatment is THE logical thing to do. In fact, performing NO genotyping would, in view of current knowledge, be completely unethical. Since the alternative for tamoxifen are the much more expensive aromatase inhibitors, cost effectiveness of this approach is clear-cut. We should therefore not withhold breast cancer patients this valuable analysis.

PC1 - 2

Pharmacogenetics in predicting anti-cancer therapy response – contras

Quirke Philip

University of Leeds, Pathology and Tumour Biology, Leeds, United Kingdom

Corresponding author: p.quirke@leeds.ac.uk

Background: Prediction of therapeutic response is critical to progress in personalized medicine. Pharmacogenetics is generally regarded as the study or clinical testing of genetic variation that gives rise to differing responses to drugs whereas pharmacogenomics refers to somatic mutations in tumoral

DNA leading to alteration in drug response. There are issues that must be considered in both areas and these will be addressed including the importance of study design e.g. use of clinical trials, prognostic *vs*. predictive markers, adequate hypothesis generating and validating studies, small numbers etc. The lack of rigour of approval agencies and their changing standards does not help the field.

Pharmacogenomics has come of age and is identifying genetic markers in pathways that identify responders, non responders, the development of induced tumour resistance and is shedding light on mechanisms of drug failure. There is rapid translation from lab to clinic and many examples exist e.g. Her-2 amplification and Herceptin response, Ki-ras and anti EGFr non response, selective mutation of the external domain of EGFr and resistance to anti-EGFr antibodies.

The study of genetic variation in pharmacogenetics has yielded less value, controversial results and few tests are in routine clinical use in cancer.

Results: Data on SNPs cardiovascular disease and colorectal cancer SNP's will be shown and UGT1A1 discussed.

Conclusion: Pharmacogenomics is already here and providing important clinical information. Pharmacogenetics has yet to earn its place in the clinic.

PC2 - Laboratory screening for thrombophilia - some important dilemmas

PC2 - 1

Pro's and con's of thrombophilia testing: pro's

Palareti Gualtiero

University Hospital of Bologna, Angiology and Blood Coagulation, Bologna, Italy

Corresponding author: gualtiero.palareti@unibo.it

Heritable thrombophilia is associated with a tendency to venous thromboembolism (VTE), with a risk that is greater in case of antithrombin, protein C or S deficiency, and lower in presence of factor V Leiden or Prothrombin mutation. The latter are however are extremely frequent, while the former are relatively rare. Altogether their prevalence is about 8-10% in the general population, a situation that nudges up the probability of carrying multiple alterations, with a further increase in thrombotic risk up to 20-fold. Testing in unselected patients with VTE is not indicated. However, testing selected patients may give important information on the risk of recurrences after completion of the standard first course of anticoagulant treatment and help clinicians to decide on the optimal duration of anticoagulation in the individual patient. Patients with a first unprovoked VTE (especially if young), with recurrent VTE, with VTE during pregnancy/puerperium or during contraceptive or replacement hormone therapy, with thrombosis in unusual sites, are candidate for testing. Other subjects can be examined: asymptomatic subjects, with a family history of VTE and a first-degree relative with thrombophilia diagnosis, before important conditions at risk such as pregnancy or hormone therapy. Thrombophilia has also been claimed as possible risk factor for pregnancy complications; women with a history of pregnancy loss, recurrent or late in pregnancy should be tested. Testing should be patient-specific, because results are affected by many preanalytic variables, such as pregnancy or hormone therapy, the acute phase of VTE and drugs used for anticoagulation.

PC2 – 2

Pro's and con's of thrombophilia testing: con's

Abstract not provided.

PC3 - Screening for bowel cancer - FOBT, FIT and/or FLEXI-SIG?

PC3 - 1

Laboratory screening for colorectal cancer

Halloran Stephen

National Health Service, Bowel Cancer Screening Programme, Guildford, United Kingdom

Corresponding author: s.halloran@nhs.net

Colorectal cancer is the second most common cause of cancer deaths in Europe and probably the most common cause in men who do not smoke! Whilst tumour markers largely play second fiddle in front-line cancer diagnosis, the clinical niche for faecal occult blood testing (FOBT) has finally been recognised after decades of making an uncertain contribution to the process of diagnosing colorectal cancer.

Two years ago the EU published guidelines on population screening for colorectal cancer and described how using a simple test can save the lives of many destined to die from colorectal cancer.

The diagnostic attributes of guaiac were recognised in 1864 after it was shown impotent in the treatment of syphilis. Reincarnation of FOBT equipped it for small volume analysis but not for population screening. The practical and analytical deficiencies of guaiac FOBT has thrust faecal immunochemical testing for haemoglobin (FIT) into pole position as the EU-recommended test for colorectal cancer screening.

Whilst Poland and Germany maintain a commitment to colonoscopy, the world is now embracing the evidence behind biomarker population screening.

Can the international biochemistry community guarantee consistent, high quality, professionally-led services for this major public health initiative? As this Cinderella analyte moves centre stage, we are challenged to develop safe systems of analysis, adequate sample preservatives, traceable calibration and internationally agreed units of reporting.

The presentation reviews the strengths and weaknesses of FOBT, reveals its impact in the UK screening programmes and highlights the opportunities and challenges for the future.

PC3 - 2

Endoscopic screening for bowel cancer

Atkin Wendy

Imperial College London, Department of Surgery & Cancer, London, United Kingdom

Corresponding author: w.atkin@imperial.ac.uk

Endoscopic screening of the colon is offered opportunistically in most high-risk countries, but few countries have implemented organised screening programmes. This is probably because there has been an absence of high-quality evidence in the form of randomised controlled trials (RCT). During the past three years, however, three trials have demonstrated the long-term efficacy, safety, and cost-effectiveness of flexible sigmoidoscopy (FS) screening. Two showed that a single FS, offered between the ages of 55 and 64, causes a reduction in colorectal cancer incidence that lasts at least 10 years. The other trial showed a lasting benefit from two FS screenings. England is the first country to adopt a national programme based on a single FS offered at age 55.

FS has been criticised as being 'necessarily short-sighted' since it can reliably examine only the rectum and sigmoid colon. Colonoscopy can examine the whole colon but is a more onerous procedure, requiring full bowel preparation and often sedation, leading to a commitment of at least 48 hours in preparation and recovery time. FS, by contrast, requires only a single, self-administered enema and no sedation. Moreover, there are no RCTs examining the incremental benefit in terms of reduction in incidence and mortality of right-sided colon cancer to justify the use of colonoscopy; two RCTs have recently started, but they are not expected to report for at least 10 years. Other methods of screening the right colon include faecal occult blood testing and new DNA-based stool tests, which are currently under research.

PC4 - Does accreditation improve patient outcome?

PC4 - 1

Laboratory accreditation - where is the real benefit for the patients?

Vukasovic Ines

Medical School University Hospital Sestre Milosrdnice Zagreb, Croatia, Clinical Institute of Chemistry, Zagreb, Croatia

Corresponding author: ines.vukasovic@gmail.com

Accreditation is widely used in developed countries to encourage or enforce improvements in the quality and reliability of laboratories. The prime aim of accreditation is to prove, by objective evidence, its competence to provide a medical diagnostic service to its customers, including healthcare staff and patients. Patient management decisions are based on laboratory data. Medical diagnostic services is far broader than just performing analysis of a certain measurand and include a whole range of activities: advising physicians on selection the most appropriate tests for the specific diagnostic problem, instructions on sampling and pre-analytical variables, testing by analytical methods, reporting and interpreting test results in the clinical context. The ISO15189 standard is recognized as a reliable indicator of laboratory technical competence and helps laboratories to provide the better services for the patients who expect that all tests are carried out according to the highest quality principles. If these tests are not available or are inaccurate, treatment outcomes for patients are likely to be poorer, with higher mortality and more frequent illness. Diagnostic testing involves multistep processes which could potentially be a subject to multiple sources of error leading to significant variance in the accuracy of the reported result, incorrect diagnosis, inappropriate treatment, or withholding of lifesaving therapy. Laboratory should consider all potential errors that could cause the worst outcomes and prevent them. Laboratories that achieve accreditation are recognized for superior test reliability, operational performance, quality management, reduced rates of laboratory errors and ultimately contribute to better patient care.

PC4 - 2

Contra laboratory accreditation: Where is the real benefit for patients ?

Müller Mathias M.

Austrian Society for Quality Assurance and Standardisation, Head Quarter, Vienna, Austria

Corresponding author: mathias.mueller1@chello.a

Accreditation of medical laboratories is based on the ISO 15189 standards describing their competence and quality in the whole diagnostic process. Advantages and or disadvantages for a diagnostic laboratory are important questions, since accreditation and keeping the accreditation status of a laboratory are costly.

The ISO standard describes the important technical laboratory processes, following strictly self-defined quality criteria described in subjective quality handbooks. Administrative processes are implemented, new developments of analytical techniques and their use in clinical diagnostics is prohibited. The discipline of laboratory diagnostics loses its innovation within medical sciences.

During the last 5 to 10 years the structure and the competence of a diagnostic laboratory has changed fundamentally by industry driven mega laboratories thus loosing competence in analytics, pathophysiology, medical interpretation of laboratory results by focusing on economics, and accreditation rules concomitant with an administrative overkill. Accredited laboratories are mainly run by economics. Professional competence is measured on basis of keeping with the annual budget.

Modern quality management has been developed as an important field for quality managers not for patients, physicians and laboratory professionals. Searching the literature, the benefit of accreditation is not documented; the number of correct diagnosis has not increased. The diagnostic laboratory must be an integrated institution in clinical medicine being fundamental for the benefit of patients. These are the real goals of a medical laboratory not being measured by industry standards.

Posters

P01 - Accreditation and laboratory management

P01-01

Increasing profitability of a specialized medical biochemistry laboratory

Buljanovic V (1), Patajac H (2), Petrovecki M (3)

(1) General Country Hospital Našice, Specialized Medical Biochemistry Laboratory, Našice, Croatia
(2) Adris Group d.d., Adris Group d.d., Rovinj, Croatia
(3) Dubrava University Hospital, School of Medicine, University of Rijeka, Rijeka, Croatia, Zagreb, Croatia

Corresponding author: vikica.buljanovic@os.t-com.hr

Introduction: Laboratory's profitability is expressed as the operating profit, which is the final result of profit and loss account. Operating profit may be increased by organizational changes, i.e. by monitoring all laboratory expenses and processes.

Methods: The annual profitability of the specialized Medical Biochemistry Laboratory at General County Hospital in Našice, Croatia, in the study period 2007-2010 was evaluated using the profit and loss account, which included three basic elements: revenue, expenses, and their difference. Revenues were realized from reimbursements for laboratory tests performed, and expenses included all expenditures required for the realization of the annual revenue.

Results: The Laboratory's operating profit in 2007 was HRK 719,926, and the operating margin was 12%, (profit expressed as percentage), showing that the laboratory made profit and that, after the deduction of all operating expenses per 100 units of revenue, it retained 12 units of profit from performing its basic operating activities. In 2008, the operating profit increased to HRK 3,415,269 and operating margin increased to 39%, which is a 4.7-fold increase in comparison with the previous year. In 2009 and 2010, operating profits were HRK 3,049,245 and HRK 3,149,922, respectively, and the operating margin was 36%.

Conclusion: The analysis of the operating business performance of the specialized Medical Biochemistry Laboratory at General County Hospital in Našice showed that laboratory's profitability could be increased and maintained only by implementing organizational changes, without any technological investments. This finding could help other laboratories in Croatia to increase their profitability.

P01-02

Six sigma in clinical laboratory: analytical process management – focus on reduction of response time

Ribeiro R, Sousa MJ, Sousa JG, Sousa G

Centro Medicina Laboratorial DR. Germano de Sousa, Quality, Lisbon, Portugal

Corresponding author: rribeiro@cm-lab.com

The philosophy of Six Sigma quality can be seen as a strategy that achieves perfection in products and processes. This holistic view was a new tool for the Clinical Laboratory, which in the context of business competitiveness concerns the relentless pursuit of perfect products by establishing an organizational advantage in various aspects. The product of the clinical laboratory is the result of quality from the service until the delivery of the report of the results. From the standpoint of the user, there are two perceived characteristics: accurate and unambiguous results, and the time of delivery. The Six Sigma project developed in Centro Medicina Laboratorial Dr. Germano de Sousa can bring great benefits to the analytical process, in the management of the analytical process of clinical laboratory, focusing on response time, and maintaining the quality of analytical results. According to the studies of reproducibility and repeatability, along with sigma metric as an index of performance we want to standardize the analytical repetitions performed per parameter to confirm the results, thus reducing the process time and reducing costs as the cost of using / technical occupation, additional expense of reagents and possible repeated harvesting by insufficient amount of biological sample. The use of a Six Sigma strategy in the clinical laboratory, not just being used as a metric for performance analysis, but in the context of case management provides a stand-

ardization of a complex and multidisciplinary, aligns the goals of quality and costs, focusing on customers and the health system´s financial organization.

P01-03

Development of the balanced scorecard of a software program for the management of quality indicators

De La Torre Fernandez J, Soriano Bueno G, Cazalla Martin F, Navajas Luque F, Rodriguez Peña F, Guzman Gonzalez A

Hospital Comarcal De La Axarquia, Velez-Malaga, Laboratory, Malaga, Spain

Corresponding author: gsorib@gmail.com

Background: Indicators related to laboratory processes represented in a balanced scorecard (BCS) can be obtained in an automated, fast and effective way through the Omnium software, for the continuous improvement of the quality.

Materials and methods: (17) indicators are calculated monthly from variables (70) registered daily in the tab of each patient of the laboratory computer system and indicate a resulted incidence. Areas, processes, fields and aggregators are defined in the BCS module of Omnium. The computer application Omnium (Roche) acts as DataWareHouse selecting, filtering and transforming data from Omega (Roche) and generating results from each indicator with its goal classified by colors. A browser displays the BCS in aggregators, fields, processes, areas and indicators, and a BCS tree graphically represents it to obtain the requested information, to study the evolution.

Results: 17 indicators are used: 6 of quality of the application, 4 of extraction, 4 of processing and transport, 1 of reception, 1of validation time and 1 of response time. The objective of each preanalytical indicator is < 0.3%, alarm indicator between 0.8 and 1.3, and danger indicator >1.3%. In the period January-May of 2012 all our indicators were below the target except "Samples not received", which value was 1.35.

Conclusions: With "Omnium", we obtain indicators of monthly automated extraction framed in a BCS organized in variables, indicators, areas, processes and fields. Staff knows their monthly indicators, evolution and goals for the following month. The browser and the BCS tree allow a better monitoring of the indicators evolution.

P01-04

Artificial urine – possibility or a good try

Culej J (1), Nikolac N (2), Krajačić-Karas G (1), Šturm D (1)

(1) Sestre Milosrdnice University Hospital, Department of transfusiology and haemostasis - Clinic for tumors, Zagreb, Croatia
(2) Sestre Milosrdnice University Hospital, Clinical Institute of Chemistry, Zagreb, Croatia

Corresponding author: jelena.culej@gmail.com

Background: Based to our knowledge, external quality control assessment for urinalysis in most schemes is organized through sending pictures of urine sediment into laboratories. Although useful in testing of element recognition, this approach doesn't cover the whole process of urine sediment preparation. The aim of our study was to prepare artificial, low infective sample in order to test whole process of urine sediment analysis.

Materials and methods: We suspended small amount of human blood, epithelial cells from buccal mucosa and baker's yeast in saline fluid. This mixture was distributed into 13 samples and given for sediment analysis to a 13 professionals into 4 laboratories on the same day.

Results: As we expected, most of professionals managed to recognize all elements added into saline. Overall agreement for erythrocytes was 13/13, for epithelial cells 12/13, for leukocytes 10/13 and for yeasts 9/13. Relatively low agreement on yeast recognition might be due to growth of cells in samples that were examined last and also because of similarity of these elements to erythrocytes. Raters that didn't find any leukocytes are from laboratory that reported smaller amount of urine used for

analysis. Some of raters reported artefacts and salts that were not present in the samples. Also, they were confused with morphology of yeasts and complained about morphology of erythrocytes.

Conclusion: This approach has potential to develop into quality control of the total process of urine sediment analysis. However, because the transport conditions could influence composition of samples it could be applied locally.

P01-05

Accreditation according to standard UNE-EN-ISO 15189: 2007. Evaluation of a program in biochemistry

Cazalla Martin F, Soriano Bueno G, De La Torre Fernandez J, Navajas Luque F, Sanchez-Montes Moreno S, De La Torre Navarrete J

Hospital Comarcal De La Axarquia, Velez-Malaga, Laboratory, Malaga, Spain

Corresponding author: gsorib@gmail.com

Background: Accreditation of laboratories and the improvements in their results have been running quality systems for the continuous assessment and long-term accuracy of measure procedures by the results of the external quality control (CCE).

Materials and methods: We collected annual results of studied analytes sent by the Centre of Calculation of External Quality Control. The CCE has been processed monthly during 2011 in a Modular Cobasc711 where 23 parameters have been evaluated. These data allow us to know for each analyte: number of total data, deleted by aberrant, average and standard deviation. We studied rates of standard deviation, the total Error (%) according to desirable specification based on biological variation and performance techniques using the z-score.

Results: 276 results annually obtained for 23 different techniques included in the external quality control program have been evaluated. According to the standard deviation rate (IDS) we have established three groups: <1 IDS, 211 results (76.45%); 1-2 IDS, 60 (21.74%); 2-3 IDS, 5 (1.81%). An acceptable IDS is considered to be +/- 2. We obtained a 98.19% of acceptable results and a 1.81% of non acceptable results. 18 techniques (78.26%) obtained a specification of excellent quality by calculating the total Error (%) and 5 (21.74%) a desirable specification. In terms of performance, 14 parameters (60.87%) presented a satisfactory z-score rate (Pz < 2).

Conclusions: During 2011, the 98.19% of the results were acceptable (+/- 2IDS). All parameters were below the desirable Total Error according to specifications based on biological variation. 14 studied parameters (60.87%) obtained a satisfactory performance (Pz score<2).

P01-06

Information systems implementation: impact on the pre analytical phase of laboratory work

Fuček M, Šrenger V, Rako I, Zekušić M, Alpeza Viman I, Sertić J

Clinical Hospital Centre (KBC), Zagreb, Croatia, Department of Laboratory Diagnostics (KZLD), Zagreb, Croatia

Corresponding author: mfucek@kbc-zagreb.hr

Background: Laboratory Information System (LIS) BioNET which has been applied at the Department of Laboratory Diagnostics (KZLD) Clinical Hospital Centre (KBC) Zagreb since 2006 is upgraded with an electronic module that provides a record of nonconforming samples. Before the upgrade, every pre analytical error was paper-based and the department from which sample was sent was informed by phone. Electronic reporting of pre analytical errors is easier and simpler. Information about error is immediately distributed by HL7 communications protocol to hospital information system and accessible for a clinician.

At the same time, KBC Zagreb was introduced with an integrated hospital and business information system (BIPSI). The clinical departments have started with assigning electronic laboratory referrals

and labeling samples with barcode. HL7 communications protocol automatically transmits all requests to LIS which generates the barcode label.

Materials and methods: Laboratory Information System (LIS) BioNET, hospital and business information system (BIPSI), HL7 communications protocol

Results: In this way the sample is identified only once - at the department where is taken. The time of pre analytical phase is reduced as well as incorrect labeling.

Conclusions: Results of the implementation of these information systems are reduced number of pre analytical errors, faster elimination of recorded errors and consequently better patient care.

P01-07

Structured handoff at shift change in a clinical laboratory

de Jonge N, Ballieux B, Schenk P, Cobbaert C

Leiden University Medical Center, Department of Clinical Chemistry, Leiden, Netherlands

Corresponding author: n.de_jonge@lumc.nl

Background: It is estimated that 60-70% of medical decisions are based on clinical laboratory results. The laboratory therefore plays a crucial role in patient safety. As miscommunication during shift handover is a well-known risk in other high-risk environments, we developed a structured handoff procedure.

Materials and methods: We implemented a structured handoff for the clinical chemist on call. On-call shifts last for one week (24/7). The clinical chemist on call is in-house during office hours and can be reached by mobile phone outside office hours. The handoff was based on best practices in other high-risk environments, such as air traffic control and nuclear power plants. The following procedure was developed:

- The incoming clinical chemist assesses the status of the lab.
- The outgoing clinical chemist prepares the handoff.
- In case of calamities the handoff is delayed.
- Handoffs are not to be interrupted, and take place in a separate room.
- Handoff is an equal responsibility for incoming and outgoing clinical chemist.
- At every handoff a checklist (current state of laboratory staffing, IT, technical and analytical issues, non-conformities, service level, patient cases for handoff, and cases for continuous education) is used.

Results: The use of a structured checklist provided a continuous and thorough overview of the status of all relevant laboratory aspects to the clinical chemist on call.

Conclusions: The structured handoff resulted in several improvements: shorter duration and less escalation of IT, technical and analytical quality issues, improved service towards clinicians and contribution to continuous education.

P01-08

Effects of laboratory automation – report from a university hospital subjected to great changes

Skinningsrud A

Akershus University Hospital, Department of Multidisciplinary Laboratory Medicine and Medical Biochemistry, Lørenskog, Norway

Corresponding author: anders.skinningsrud@ahus.no

Background: Akershus University Hospital in the greater Oslo area has 758 somatic and 345 psychiatric beds. From 2011, the population served increased with 140,000, and the uptake area is currently 455,000. Approximately 40% of the hospital's laboratory analyses are for the primary health care services. Automation of most laboratory analyses and the preanalytical phase was implement-

ed as we moved into a new hospital building in 2008.

Materials and methods: Our system for nearly total laboratory automation implemented in 2008, has automatic centrifugation, enGen and Vitros chemistry instruments from Ortho Clinical Diagnostics, immunochemistry from Beckman Coulter Inc. and a hematology trac from Sysmex Corporation. Workload and turnaround time (from receipt of the sample to reporting of results) were recorded.

Results and conclusions: Automation led to reduced turnaround times for most in-house analyses and enabled handling of a 30% increase in workload without additional staff. Previously most of the primary healthcare samples were analyzed the following day. After automation they were analyzed the same day. Turnaround time for primary healthcare samples was at least 12 hours less after automation. Automation enabled allocation of more resources to research and setting up new analyses. The sudden increase in hospital workload had not been possible without automation of laboratory procedures.

P01-09

Rationalization of spenditure in laboratory department in primary health care system in Serbia

Milatovic Jezdic V (1), Mitrovic J (2)

(1) Primary Haelth Care Savski Venac, Diagnostic, Belgrade, Serbia
(2) University Children's Hospital, Laboratory, Belgrade, Serbia

Corresponding author: vmilatovic@yahoo.com

Background: Clinical biochemistry is a science which task is in vitro analysis human biological materials in order to determine potential risk of illness, to confirmed or exclude presence of illness, to monitor progression of illness, for therapeutic drug monitoring, or to predict outcome of the therapy. Number of tests is in progress every year and that has an impact on dynamic of organizational changes in the laboratory. Significant increase of number of tests leads to increase of financial resources which should be spent on clinical-biochemistry laboratories. During the time of limited financial recourses in which we are streaming to equally availability of health care system and increasing quality of service, it is necessary to make a balance between real clinician need for test request, quality of the result and spenditure of financial resources.

Resume: Rationalization of spenditure is observed from these aspects:

- laboratory as a service with IT user requests
- with clinician requests
- communication between clinicians

Laboratory as a service which is fulfilling user request is monitored from the management aspect with procedures, orders, quality control system. In this segment, laboratory work processes are introduced and how it is possible to provide rationalization in the laboratory.

Conclusion: By using these aspects, rational use of laboratory diagnostic services and rational use of resources in the treatment of patients with different diagnoses can be achieved, as well as the quality improving of health care and services that are provided to the patient.

P01-10

Microsoft Access database management of laboratory accreditation

Štefanović M, Vukasović I, Nikolac N, Ćelap I

Sestre Milosrdnice University Hospital, Clinical Institute of Chemistry, Zagreb, Croatia

Corresponding author: mstefan6@gmail.com

Laboratory accreditation management is a complex task producing many documents that need to be well and systematically organized. We build

a system organized through several Microsoft Access database applications that produce, organize or simply manage different types of accreditation documents. Frontend applications are available on every laboratory computer and access permissions are defined through login system. The database data are located on central file share server.

Document hierarchy and managing are performed trough searchable Documents database that keeps all documents organized by types, accreditation fields, and laboratory organizational units. Every document in a database is easily accessible through Intranet access.

Laboratory equipment is organized through another application database that enables managing of every laboratory part of equipment, including planning of periodically equipment calibration.

Laboratory tests data are managed through Database of measurement procedures, which also serves for reporting of yearly test reevaluations and is a data serving application for laboratory web pages for Internet available test catalogue.

Laboratory personnel is managed through database which contains possibilities of yearly appraisement for every employee, managing education, personnel test performance skills and equipment training.

Nonconformities and maintenance of laboratory equipment are managed through another database which replaces the need for paper form evidence for almost any type of information that needs to be collected on daily or periodically basis.

Paper forms and paper documents are drastically reduced after 5 years of implementing such informational system. Retrieving the statistical report for almost any type of collected data is performed by a button click.

P01-11

Do we meet quality specifications based on biological variation?

Celap I, Vukasovic I, Vrkic N

Clinical Institute of Chemistry, University Hospital Center Sestre Milosrdnice, Zagreb

Corresponding author: ivana.celap@gmail.com

Background: Responsibility of the clinical laboratory professionals is to assure the highest possible quality of the reported results. Consensus recommendation on the quality targets in laboratory medicine, which was released after the Stockholm Conference, supports the hierarchy quality model. In this concept, a model higher in the hierarchy should be preferred over lower one. Since in our laboratory the analytical performance is evaluated according the manufacturer recommendation, the lowest model in hierarchy, we aimed to compare our analytical coefficients of variation (CVa) against criteria for imprecision based on biological variation (CVb).

Materials and methods: Imprecision of 20 biochemistry parameters was monitored by analyzing two levels of commercial quality control material, three times per day, over one year. CVa obtained for each parameter was evaluated against quality specifications based on biological variation.

Results and conclusion: Comparison against biological variation was as follows: five parameters met optimum quality specifications CVa<0.25 CVb (bilirubin total and conjugated, CRP, CK and lactate); seven parameters met desirable quality specifications CVa<0.50 CVb (AST, ALT, amylase, CK-MB activity, lipase, urea, TnT) and three parameters met minimum quality specifications CVa<0.75 CVb (glucose, potassium, LD). The rest of the monitored analytes did not meet quality specifications based on biological variation. The results showed that it would be possibly better to adopt the highest model with more stringent criteria.

P01-12

Patient safety in the clinical laboratory: an analysis of patient/specimen identification errors

Cuadrado-Cenzual M (1), Maciñeira F (2), Alvarez C (2), Ortega I (2), Alguacil A (2), Arroyo M (2)

(1) Hospital Clínico San Carlos, Analisis CLinicos.U.Calidad, Madrid, Spain
(2) Hospital Clinico, Analisis CLinicos, Madrid, Spain

Corresponding author: mcuadrado.hcsc@salud.madrid.org

Background: Patient identification and specimen labeling represent one of the most critical area in patient safety and is an increasingly visible mission for clinical laboratories. The aim of this work is to assess patient identification and specimen labeling improvement after implementation projects using longitudinal statistical tools.

Materials and methods: Patient/specimen identification errors were categorized by a multidisciplinary health care team. They were grouped into 3 categories: A: specimen/requisition mismatch, B: unlabeled patient identifications, C: misidentification patient. These types of identification errors were compared preimplementation and postimplementation for 3 patient safety projects: 1) Development of Identification Patient and Specimen Process to follow by all the professionals implied; 2) reorganization of phlebotomy; 3) introduction of an electronic event reporting system. We use trend analysis and student t-test.

Results: Of 46,632 total requests analyzed, requisition mismatches, unlabeled patient identification and misidentification patient represented 1.6/10,000, 5.8/10.000, and 4.1/10.000 of errors, respectively. Student t-test showed a significant decrease in the two most serious errors, mislabeled specimens ($P < 0.001$) and misidentification patient ($P < 0.001$) when compared to before implementation. Trend analysis demonstrated decreases in all 3 error types for 18 months.

Conclusions: The applied strategies have demonstrated to be effective in the improvement of the identification of the patient in the analytical requests. However, we must continue working in this strategy, with all the implied professionals and trying to reach the objective of which the 100% of the requests they are identified correctly.

P01-13

Risk management in laboratory. Failure models and effects analysis of identification patient errors

Cuadrado-Cenzual M (1), de Gracia Y (2), Maciñeira F (2), Alvarez C (2), Guessous A (2), Arroyo M (2)

(1) Hospital Clinico, A.Clinicos. Unidad Calidad, Madrid, Spain
(2) Hospital CLinico, Analisis Clinicos, Madrid, Spain

Corresponding author: mcuadrado.hcsc@salud.madrid.org

Background: Patient and specimen identification errors are a key concern for patient safety. Failure Models and Effects Analysis (FMEA) is a tool that prospectively identifies process steps at risk for patient safety errors and permits proactively design strategies for improvement. The aim of this work was: Establish the weighted risk factors for identification patient and specimen phlebotomy process using FMEA.

Materials and methods: A multidisciplinary health care team was working in this area, reviewed phlebotomy and specimen collection process. Steps included the following: 1) diagramming the process, 2) brainstorming potential failure modes and their effects, 3) prioritizing failure modes, and 4) identifying root causes of failure modes.

Results: FMEA identified several factors that contribute to a higher likelihood of error than indicated for laboratory phlebotomy draws. The highest risk process steps were comparing preprinted, barcoded sample labels with Patient Identification and comparing them with information order sheets (RPN scores for both, 280). Moderate risk was checking patient identification for 2 identifiers and prelabeling specimens were also high risk, with RPN scores of 175. The distractions and tiring may also contribute to increased risk not explicitly noted in process steps.

Conclusions: FMEA indicated that patient identification error was the most risk-prone process step. It validates the importance of patient identification in health care. The FMEA is a very effective tool as risk management, in identifying where risk existed for patient–specimen identification errors. It also helped to create a clear partnership with other health care professionals.

P01-14

Risk management in medical laboratory. Appliyng root cause analysis of patient and specimen errors

Cuadrado-Cenzual M (1), Maciñeira F (2), Torrubia B (2), Guessous A (2), de Gracia Y (2), Arroyo M (2)

(1) Hospital Clinico, A.CLinicos. Unidad de Calidad, Madrid, Spain
(2) Hospital Clinico, Analisis CLinicos, Madrid, Spain

Corresponding author: mcuadrado.hcsc@salud.madrid.org

Background: Mislabeled laboratory specimens and errors in patient identification represent one of the problems with greater index of factor of risk in causing harm in the patient, such as repeat phlebotomy; repeat diagnostic procedure; increase of demand others diagnostic procedures, delay in a necessary surgical procedure or diagnostic. The aims of the present work was to identify system vulnerabilities in specimen collection, processing, analysis, and reporting associated with patient misidentification involving the clinical laboratory.

Materials and methods: We reviewed multiple cases of incorrect patient identification on laboratory specimens. Specimen errors were categorized by a multidisciplinary health care team. A qualitative analysis was performed on 31 root cause analysis reports. Data were categorized by the 3 stages of the laboratory test cycle.

Results: Patient misidentification accounted for 25 of 31 adverse events. 23 misidentification events occurring in the preanalytic phase the causes were: specimen mislabeling during collection (N = 20), laboratory tests were ordered for the wrong patient (N = 2) misinformation from manual entry on laboratory forms (N = 1). There was one event in the postanalytic phase in which results were reported into the wrong patient medical record (N = 2).

Conclusions: Patient and or specimen misidentification in medical laboratory were due to a limited set of causal factors in all 3 phases of the test cycle. A focus on these factors will inform systemic mitigation and prevention strategies.

P01-15

IRATA - program for managing off-balance sheet warehouse

Zaninović L (1), Tiljak-Srna V (2), Alpeza Viman I (1), Fuček M (1), Sertić J (1)

(1) Clinical Hospital Centre (KBC), Zagreb, Croatia, Department of Laboratory Diagnostics (KZLD), Zagreb, Croatia
(2) IRATA software & hardware, Zagreb, Croatia, IRATA software & hardware, Zagreb, Croatia, Zagreb, Croatia

Corresponding author: ljzanin@kbc-zagreb.hr

Background: The Department of Laboratory Diagnostics (KZLD), Clinical Hospital Centre (KBC) Zagreb has introduced a validated result that represents the total consumption of reagents required to make an analysis of certain parameters. For this reason, the company IRATA has made a program for off-balance sheet warehouse for managing goods and materials.

Materials and methods: The protocol for stock management software IRATA.

Results: Before introducing IRATA program, keeping goods in stock was done in Excel tables. After introduction of the IRATA program, traceability for each reagent was obtained from entering the warehouse to the issuance to the analyzers by quantities and material costs and also by the serial numbers and expiry dates. IRATA program allows simple and rapid inventory at any time, trace consumptions by reagents for each clinical unit in KZLD or by the suppliers of reagents. The program is a useful tool

for daily, monthly or yearly reports on the consumption of reagents and validated results.

Conclusions: By introducing IRATA program for managing stock, recording of overall process within all clinical units in KZLD was significantly facilitated. The inventory of goods was significantly simplified, and for accreditation (ISO 15189), essential reagents traceability by serial numbers and expiry dates of arrival at the laboratory to its application to the analyzers was obtained.

P02 – Cardiovascular diseases

P02-01

CETP, LDL particle size and intima media thickness in patients with coronary heart disease

Tosheska-Trajkovska K (1), Labudovic D (1), Jovanova S (2), Jaglikovski B (3), Alabakovska S (1)

(1) Medical Faculty-Skopje, Department of medical and experimental biochemistry, Skopje, Macedonia, TFYR
(2) University Cardiology Clinic, Department of ultrasonography, Skopje, Macedonia, TFYR
(3) AVICENA Laboratory-Skopje, Department of medical biochemistry, Skopje, Macedonia, TFYR

Corresponding author: tosheskatrajkovska@gmail.com

Background: Cholesteryl ester transfer protein (CETP) plays a key role in reverse cholesterol transport and high density lipoprotein (HDL) metabolism. Predominance of small, dense LDL particles is associated with an increased risk of atherosclerosis and coronary heart disease (CHD).

Aim: to determine the potential relationship between the CETP concentration and low density lipoprotein (LDL) particle size and their association with intima media thickness (IMT) in patients with CHD.

Materials and methods: Lipid parameters, CETP concentration and LDL particle size were determined in 100 healthy subjects (control group) and in 100 patients with CHD, aged 43 to 77 years. Plasma CETP concentrations were measured by an enzyme-linked immuno-sorbent assay with two different monoclonal antibodies. LDL subclasses were separated by nondenaturing polyacrilamide 3-31% gradient gel electrophoresis.

Results: CETP concentration was higher in patients compared to controls (2.02 ± 0.75 mg/mL *vs*. 1.74 ± 0.63 mg/mL, $P < 0.01$). Mean LDL particle size (nm) was significantly smaller in patients than in controls (24.5 ± 1.1 *vs*. 26.1 ± 0.9; $P < 0.001$). There was no relation between LDL size and CETP concentration ($r = -0.18$, $P = 0.072$). Age, diastolic blood pressure, CETP concentration and LDL particle size were independent factors for determining IMT by multiple linear regression analysis. They accounted for 35.2 % of the observed variability in IMT.

Conclusions: CETP concentration and LDL particle size were independent factors for determining IMT. CETP might play a role in determining lipoprotein distributions, but did not seem to be the sole factor in the formation of small LDL particles.

P02-02

NT-proBNP in anthracycline-induced cardiotoxicity in children

Grzunov A (1), Leniček-Krleža J (1), Obuljen J (1), Žižić V (1), Stepan-Giljević J (2), Kapitanović S (3)

(1) Children's Hospital Zagreb, Department of Laboratory Diagnostics, Zagreb, Croatia
(2) Children's Hospital Zagreb, Department of Haematho-oncology, Zagreb, Croatia
(3) Ruđer Bošković Institute, Department of Molecular Medicine, Zagreb, Croatia

Corresponding author: anagrzunov@gmail.com

Background: Anthracyclines (doxorubicin, daunorubicin and idarubicin) are highly efficacious antineoplastic agents for various malignances in children but their usefulness has been limited by cardiotoxicity causing cardiomyopathy and heart failure. The aim of this study was to assess the diagnostic accuracy of N-terminal-prohormone brain natriuretic peptide (NT-proBNP) in recognizing anthracycline related cardiotoxicity in children. **Materials and methods**: Serum levels of NT-proBNP

were measured by electrochemiluminescence immunoassay (ECLIA) on the Cobas e 411 analyser (Roche Diagnostic, Mannheim, Germany).

Results: We included 32 patients with median age of 15 years, who received anthracyclines in their chemotherapy. All patients had undergone cardiac evaluation that included electrocardiography and echocardiography. Toxicity was assessed according to the National Cancer Institute (NCI) Common toxicity criteria (version 2.0). With cut off of 125.0 pg/mL, sensitivity of NT-proBNP was 55.5% and specificity only 40.0%. The area under the receiver operating characteristic (ROC) curve was 0.548 (95% confidence interval (CI): 0.363-0.724) and odds ratio 0.83. Positive predictive value was 83.3 (95% CI: 58.6-96.4) and negative predictive value 14.3 (95% CI: 1.8-42.8). There was no statistical difference between serum NT-proBNP levels of the patients with normal and abnormal echocardiographic and electrocardiographic findings (P = 0.736).

Conclusions: Due to specificity and sensitivity of NT-proBNP, present study indicated that electrocardiographic and echocardiographic follow-up is more reliable than serum NT-proBNP levels for detecting cardiotoxicity. Further investigations in finding non-invasive and practical method for monitoring to identify cardiac damage at subclinical level are necessary.

P02-03

Cardiac biomarkers for monitoring patients with percutaneous coronary intervention

Skitek M (1), Kranjec I (2), Jerin A (1)

(1) University Medical Centre Ljubljana, Institute of Clinical Chemistry and Biochemistry, Ljubljana, Slovenia
(2) University Medical Centre Ljubljana, Department for Cardiology, Ljubljana, Slovenia

Corresponding author: milan.skitek@kclj.si

Background: The purpose of the study is to monitor glycogen phosphorylase isoenzyme BB (GPBB), CK-MB mass and troponin I (TnI) from the injured myocardium after elective percutaneous coronary intervention (PCI), in correlation with ischemic incidents.

Materials and methods: Twenty-three consecutive patients undergoing elective PCI with baseline values of CK-MB mass and TnI below the upper limit of normal (ULN) were included in the study. Baseline blood samples and two more after the PCI (3 and 24 hours) were taken. The significance of cardiac markers was evaluated based on ischemic incidents after PCI. Logistic regression analysis was used to predict ischemic incidents after PCI based on increased values of the biomarkers.

Results: An overall comparison of the biomarkers of 18 patients without and 5 patients with ischemic incidents displayed significant differences only for the baseline GPBB and CK-MB mass 24 hours after PCI (P = 0.019 and P = 0.048). Ischemic incidents were able to be predicted independently only based on overall CK-MB mass measurements (OR = 1.68, P = 0.041) and particularly GPBB at baseline (OR = 1.90, P = 0.008) and CK-MB mass 24 hours after PCI (OR = 2.11, P = 0.022).

Conclusions: Only significant increases in TnI were observed after elective PCI with the prediction of ischemic incidents only possible using GPBB and CK-MB mass measurements.

P02-04

Unexpectedly elevated cardiac Troponin I level in the patient without acute coronary syndrome: a case report

Hrabric Vlah S (1), Sokolić J (2), Medved I (3), Dvornik Š (1)

(1) Clinical Hospital Center Rijeka, Clinical Department of Laboratory Diagnostics, Rijeka, Croatia
(2) Clinical Hospital Center Rijeka, Clinical Department of Anestesiology and ICU, Rijeka, Croatia
(3) Clinical Hospital Center Rijeka, Department of Cardiac Surgery, Rijeka, Croatia

Corresponding author: snjezana.hrabric.vlah@gmail.com

Since cardiacTroponin I (cTnI) - assay is leading test in diagnostic of myocardial infarctions, and it is used for risk assessment in patients with acute coronary syndrome as well as unstable angina, it is very important to measure its concentration accu-

rately and precisely. Here we report a case where was significant difference in the cTnI concentrations measured by three different methods, which was detected in one patient's sera during preoperative evaluation for cardiac surgery. A 76-year-old male, with diagnosed chronic ischemic heart disease, essential hypertension, mitral insufficiency and diabetes mellitus type II, was preoperatively evaluated for elective surgical myocardial revascularisation. Due to variations in the results for the cTnI concentrations (0,62; 0,13; 0,89 µg/L) during the preoperative patient's evaluation in the reported case, the question was raised about the possibility of an interference known to occur in especially rare situations. The patient was operated at the Department of Cardiac Surgery and afterwards treated at the Department of Cardiac Intensive-Care Unit. Temporal elevation and subsequent decrease in the cTnI concentrations were monitored in the Clinical Department of Laboratory Diagnostic. It was observed that results obtain with different assays are not comparable. An elevated cTnI level alone should not be the only criterion used in establishing the diagnosis. The cTnI level should be considered in conjunction with patient's clinical symptoms, the ECG changes and other available information

P02-05

Levels of oxidized low density lipoproteins (oxLDL) and IgG autoantibodies against oxLDL in the sera

Balatskaya N (1), Budzinskaya M (2), Goldina N (1), Michajlova M (2), Parsunkova K (1)

(1) International Scientific Clinical Center 'Intermedbiophyschim, Department of Clinical Chemistry, Moscow, Russia (Russian Fed.)
(2) International Scientific Clinical Center 'Intermedbiophyschim, Department of Clinical Investigations, Moscow, Russia (Russian Fed.)

Corresponding author: balnat07@rambler.ru

Background: oxLDL is believed to play a critical role in the development progression of atherosclerosis and IgG autoantibodies against oxLDL can be used both as a parameter that consistently mirrors the occurrence of oxidation processes taking place in vivo and a criterium of an active including of immune system in pathological process.

Materials and methods: The present study aims to compare serum levels of oxLDL and IgGoxLDL in patients with atherosclerosis (N = 59) and healthy volunteers (N = 11). oxLDL and IgGoxLDL were assessed by ELISA.

Results: A preview showed that levels of oxLDL and IgGoxLDL were variable both in group of patients with atherosclerosis (1) and healthy group (2) (oxLDL (ng/mL): min 0.2, max 21.4 (1); min 0.3, max 20.6 (2); IgGoxLDL (mU/ml): min 13.74, max 1485 (1); min 73.7, max 694 (2). Median values of serum IgGoxLDL were higher in the 1st than the 2nd group: 422.8 mU/mL and 292.8 mU/mL ($P < 0.005$). At the same time median values of oxLDL did not differ.

Conclusions: IgGoxLDL was found to be increased in the sera for most of patients with atherosclerosis. The data need further consideration with detail study oxidant/antioxidant system.

P02-06

The association of inflammatory mediators and transient ischemic attack

Dukić L (1), Šimundić AM (1), Martinić-Popović I (2), Lovrenčić-Huzjan A (2), Šerić V (2)

(1) Sestre Milosrdnice University Hospital Center, Clinical Institute of Chemistry, Zagreb, Croatia
(2) Sestre Milosrdnice University Hospital Center, University Department of Neurology, Zagreb, Croatia

Corresponding author: lora.dukic@gmail.com

Background: Transient ischemic attack (TIA) develops as consequence of atherosclerosis. Our aim was to evaluate inflammatory mediators and early stages of macroangiopathy and microangiopathy in transient ischemic attack, within 12 hours from the onset of symptoms. **Materials and methods**: For-

ty-five patients (22 females, range 48-90 years) with transient ischemic attack and thirty-six controls (15 females, range 55-85 years) were included in study. Serum concentrations of cellular adhesion molecules (ICAM-1), interleukin-6 (IL-6), C-reactive protein (CRP), glucose, lipid profile and sedimentation rate were determined for all participants. Macroangiopathy was assesed by color doppler measurement of carotid intima media thickness. Degree of cerebral vasoreactivity as early marker of microangiopathy was determined by transcranial doppler ultrasound measurement of breath holding index.

Results: Levels of inflammatory mediators ICAM-1, IL-6 and CRP were significantly increased (371.6 ± 161.8 *vs*. 301.9 ± 91.6 ng/L, P = 0.026 for ICAM-1, 6.76 (2.72-18.3) *vs*. 2.46 (1.5-4.61) pg/mL, P < 0.001 for IL-6 and 8.5 (6.7-14.9) *vs*. 4.5 (2.8-5.8) mg/L, P < 0.001 for CRP) in patients with transient ischemic attack. Carotid intima-media thickness was significantly higher (1.22 ± 0.17 *vs*. 0.86 ± 0.19 mm, P < 0.001) and breath holding index was significantly lower (0.67 ± 0.19 *vs*. 1.16 ± 0.35%/s, P < 0.001) in patients with TIA compared to controls.

Conclusion: Inflammatory mediator levels and carotid intima-media thickness measured within the first 12 hours after onset of TIA symptoms are higher than in controls, which together with breath holding index lower than controls indicates high risk of future stroke.

P02-07

Role of hemostatic and other markers in patients with coronary artery disease

Bozovic D (1), Dragnic S (2), Nikolic V (1)

(1) Clinical center of Montenegro, Center for clinical-laboratory diagnostic, Podgorica, Montenegro
(2) Clinical center of Montenegro, Department of cardiology, Podgorica, Montenegro

Corresponding author: bozovicdragica@yahoo.com

Background: D-dimer, fibrinogen, hs-CRP, IL-6 and other markers have considerable utility in patients with acute coronary syndrome (ACS) and congestive heart failure (CHF). Atherosclerosis is a chronic inflammatory process, and coronary thrombosis is an important determinant of prognosis in patients with acute coronary syndromes.

Materials and methods: We examined 87 patients with coronary artery disease (CAD), mean age 62 ± 7.65 years, body mass index (BMI) 28.35 ± 4.28 kg/m^2 and 50 healthy patients with similar characteristics and with normal coronary angiograms. All test subjects were on regular diet and without any serious complications. We have determined for all their general biochemical status and complete lipid profile. All biochemical parameters were determined on Abbott's Architect C 8000, fibrinogen and D-dimer on a coagulation analyzer BCS XP Dade Behring, IL-6 on DPC Immulite 2000 from EDTA plasme, and hs-CRP was determined on nephelometer BN II Dade Behring from serum. All the samples were processed fresh.

Results: The values of fibrinogen for patients with coronary artery disease (CAD) were 4.12 ± 0.83 g/L *vs*. 3.06 ± 0.67g/L (P < 0.01), D-dimer was 1.86 ± 1.22 mg/L (P < 0.001), IL-6 was 6.39 ± 3.95 pg/mL (P < 0.05); hs-CRP values were significantly higher in patients 2.5 ± 2.47 mg/L compared to control group 1.04 ± 0.17mg/L (P < 0.001).

Conclusions: These findings suggest that increased levels of D-dimer and fibrinogen are indicative of a hypercoagulable state, as found in acute coronary syndromes. Plasma D-dimer levels are strongly and independently associated with the presence of CAD. The results indicate the importance of determining hemostatic markers and other risk factors for developing coronary artery disease.

P02-08

Serum uric acid levels and its association with coronary artery disease: an Indian Cohort Study

Das S (1), Gupta SK (1), Girish MP (2), Ray PC (1)

(1) Maulana Azad Medical College, Department of Biochemistry, New Delhi, India
(2) G.B. Pant Hospital, Department of Cardiology, New Delhi, India

Corresponding author: drsaswatidas@gmail.com

Background: The role of high uric acid levels as a risk factor for Coronary Artery Disease (CAD) is highly debated. There are very few studies assessing the role of uric acid levels with increasing severity of CAD. The aim of our study was to study the association between high uric acid levels with CAD in an Indian population.

Materials and methods: 100 patients of angiographically proven CAD were studied of which 50 patients were of stable angina (Group I), 50 patients of acute coronary syndrome (Group II) (35 patients of unstable angina and 15 patients of MI) from a tertiary health center, New Delhi and a third group comprising of 50 age and sex matched healthy controls were also studied over a period of 1 year. Angiographic clinical vessel scoring was done for all patients.

Results: The mean age of the patients was 49 ± 8.8 years (84% men, 16%women). The mean uric acid values for stable angina (Group I) (7.42 ± 1.44 mg/dL) and acute coronary syndrome (Group II) (7.47 ± 1.49 mg/dL) were significantly higher in CAD patients than controls (4.75 ± 0.79 mg/dL) ($P < 0.001$). High serum uric acid values were associated with higher vessel scores indicating a more severe CAD ($r = 0.580$, $P < 0.001$).

Conclusion: Significant correlations were found between serum uric acid levels and the established risk factors of CAD and the angiographic clinical vessel score. Asymptomatic hyperuricemia is associated with both the presence and the severity of angiographically proven CAD patients.

P02-09

Matrix metalloproteases and their inhibitors in the treatment by omega-3 polyunsaturated fatty acids

Gaikovaya L (1), Kukharchik G (2), Krapivka N (1), Nesterova N (2), Khushvaktova N (2), Vavilova T (1)

(1) North-West State Medical University named after Mechnikov, Laboratory Department, Saint-Petersburg, Russia (Russian Fed.)
(2) North-West State Medical University named after Mechnikov, Cardiology Department, St. Petersburg, Russia (Russian Fed.)

Corresponding author: vtv.lab@rambler.ru

Purpose: To evaluate an influence of therapy by Omega-3 Polyunsaturated Fatty Acids (Omega-3 PSFA) on the levels of matrix metalloprotease 9 (MMP-9), pro-matrix metalloprotease 1 (proMMP-1) and their tissue inhibitor (TIMP-1) associated with left ventricular remodelling in patients with myocardial infarction (MI).

Materials and methods: 136 patients with MI were randomized on 2 groups: the 1st: 84 patients getting standard therapy, the 2nd: 52 patients getting additionally Omega-3 PSFA in doses 900 mg/day for 3 months. The levels of proMMP-1, MMP-9 and TIMP-1 were measured on the 4-5th day and over 3 months.

Results: An initial level of proMMP-1, MMP-9 and TIMP were not different in both groups. In the 1st group the levels of MMP-9 increased regarding to initial levels reaching over 3 months 361.8 (284.8; 409.0) ng/mL ($P = 0.03$), it was connected with more marked degradation of the extracellular matrix and development of postinfarction remodelling; the levels of proMMP-1 were not changed. In the 2nd group the level of TIMP-1 over 3 months after an acute MI significantly exceeded the level of TIMP-1 in patients of the 1st group and was 1121.3 (955.2; 1612.2) ng/mL ($P = 0.006$).

Conclusions: The therapy by Omega-3 PSFA influenced on the levels of matrix metalloproteases and their tissue inhibitor with probable restraining progression of the left ventricle remodelling.

P02-10

Oxidative stress in hypertension induced congestive heart failure patients in Nigeria

Akinosun O, Sule Z

College of Medicine, University of Ibadan, Chemical Pathology Department, Ibadan, Nigeria

Corresponding author: bayoakin2002@yahoo.com

Background: Congestive heart failure is a common end point of many abnormal cardiac conditions. Cardiovascular diseases have been linked with oxidative stress which provides the strongest evidence for the protective role of antioxidants. A high consumption of fruit and vegetables which are good sources of antioxidants has been associated with lower coronary risk.

Materials and methods: Changes in total antioxidant status (TAS), vitamins A, E , C levels and antioxidant trace metals (selenium, zinc, copper, manganese) were studied in the plasma of 61 participants aged between 30 and 79 years comprising 30 hypertensive subjects without heart failure (HTN),11 hypertension induced congestive heart failure subjects (CHF) and 20 non- hypertensive apparently healthy individuals (control) using spectrophotometry, HPLC and atomic absorption spectrometry (AAS) respectively.

Results: TAS and Vitamin C in CHF were significantly lower compared with controls ($P = 0.04$, $P = 0.04$ respectively). Other parameters were not statistically different.TAS in HTN was significantly lower than controls ($P = 0.01$) while vitamin E was significantly high ($P = 0.00$). Other parameters were statistically not significant. Vitamin C was significantly lower in HTN compared with CHF ($P = 0.049$). Significant negative correlation was observed between BMI and TAS, ($r = -0.47$, $P < 0.05$) as well as Age and TAS ($r = 0.03$, $P < 0.05$) in controls.

Conclusion: CHF and HTN patients have low TAS and increased oxidative stress which may aggravate the existing cardiovascular disease. Increased dietary intake of antioxidants may be beneficial.

P02-11

New way of endothelial dysfunction research: flow cytometry testing of circulating endothelial cells

Sirotkina O (1), Feoktistova V (2), Topuzova M (3), Khushvaktova Z (2), Lipunova A (2), Vavilova T (4)

(1) B.P. Konstantinov Petersburg Nuclear Physics Institute, Human Molecular Genetics, StPetersburg, Russia (Russian Fed.)
(2) I.I. Mechnikov North-West State Medical University, Cardiology, StPetersburg, Russia (Russian Fed.)
(3) I.I. Mechnikov North-West State Medical University, Neurology, StPetersburg, Russia (Russian Fed.)
(4) I.I. Mechnikov North-West State Medical University, Clinical Laboratory Diagnostics, StPetersburg, Russia (Russian Fed.)

Corresponding author: olga_sirotkina@mail.ru

Objectives: The vascular endothelium plays a pivotal role in regulating blood flow, vascular permeability and thrombogenesis. While, endothelial dysfunction is a risk factor for cerebrovascular or cardiovascular diseases. The aim of our study was to investigate the quantity of circulating endothelial cells (EC) in patients with cerebrovascular and cardiovascular diseases and in healthy controls.

Materials and methods: We examined 63 healthy men and women (mean age 49.5 ± 1.5), 31 patients with cerebrovascular disease (mean age 70 ± 2) and 24 patients with cardiovascular disease (mean age 60 ± 2). Level of circulating EC was determined in venous blood by flow cytometry using a fluorescently-labeled antibodies specific to CD45 and CD146 as number of nucleate cells with specified size and positive binding with anti-CD146, but negative binding with anti-CD45.

Results: mean level of circulating EC in controls was 6.1 ± 0.7 cells/mL (0–25cells/mL), in patients with cerebrovascular disease 5.5 ± 1.2 cells/mL (0–33cells/mL) and in group with cardiovascular disease 6.4 ± 1.0 cells/mL (0–18 cells/mL). There were no differences in circulating EC number between studied groups. However, when we selected in cardiovascular group the patients with acute coronary syndrome due to acute myocardial infarction or unstable stenocardia and sex and age matched control, the significant differences were

found. The level of circulating EC was 7.7 ± 1.3 cells/mL and 4.9 ± 0.6 cells/mL in ACS patients and matched controls, respectively (P = 0.049).

Conclusion: the determination of circulating EC by flow cytometry may indicate the vascular wall's damage in acute stage of cardiovascular disease and serve as accessible method of clinical laboratory diagnostics.

P02-12

Correlation between two methods for determination of cardiac troponin I

Ille K (1), Spasić Obradović G (1), Mitrović J (2), Milatović Jezdić V (3)

(1) Zvezdara University Medical Centre, Laboratory of Clinical biochemistry, Belgrad, Serbia
(2) University Children's Hospital, Laboratory of Clinical biochemistry, Belgrade, Serbia
(3) Primary Health Centre Savski Venac, Laboratory of Clinical biochemistry, Belgrade, Serbia

Corresponding author: katarina.ille@gmail.com

Background: Cardiac troponin I (cTnI) is sensitive marker that reflects damage to myocardial cells, which is for years used in the Laboratory of Belgrade's University Zvezdara Medical Center. Siemens Immulite 1000 was regularly used for determination of cTnI. Aiming to improve care for our patients we have decided to examine new method for determination of cTnI by using Roche Cobas e411analyzer. The purpose of this study was to test the method before its eventual adoption in clinical routine. We compared new method for determination of cTnI with the one already applied in our laboratory.

Materials and methods: The correlation between Roche Cobas e411 cardiac troponin I (x) and Siemens Immulite 1000 cardiac troponin I (y) was performed on 100 patients (59 males aged between 43 and 86 years, and 41 females aged between 47 and 87 years) with signs and symptoms of acute myocardial infarction (AMI) admitted to our emergency department in December of 2011.

Results: The correlation was not good (y = 1.95x + 4.73; N = 100; r = 0.92). In 16 out of 100 patients (16%) the results obtained by two methods were opposite, with one method indicating presence of AMI and the other absence of it.

Conclusions: Values of cTnI obtained with two compared methods were extremely different as indicated by low coefficient of correlation and high values of intercept and slope. In significant number of patients the results obtained by two methods were opposite, with one method indicating presence of AMI and the other absence of it.

P02-13

Biomarkers of inflammation and angiographic score in patients with peripheral arterial disease

Perkov S (1), Flegar-Meštrić Z (1), Vidjak V (2), Novačić K (2), Slavica M (2), Kavur L (2)

(1) University Hospital Merkur, Institute of Clinical Chemistry and Laboratory Medicine, Zagreb, Croatia
(2) University Hospital Merkur, Clinical Department of Diagnostic and Interventional Radiology, Zagreb, Croatia

Corresponding author: sonja.perkov@gmail.com

Background: Inflammation plays an important role in the pathophysiology of peripheral arterial disease (PAD). The aim of this study was to investigate association of C-reactive protein (CRP) concentrations and the platelet-activating factor acetylhydrolase (PAF-AH) activity, as novel biomarkers of inflammation with the angiographic score in patients with PAD.

Materials and methods: The study included 110 patients with angiographically confirmed diagnosis PAD and 118 control subjects. The serum PAF-AH activity was determined by spectrophotometric assay (Azwell Inc., Auto PAF-AH, Osaka, Japan). CRP concentration was determined by high-sensitivity imunoturbidimetric assay (Beckman Coulter, OSR 6229, Ireland). The distal aorta plus 10 arteries of each lower extremity were scored on the basis of vessel lumen reduction: 1 if stenoses involved a reduction in the vessel lumen of <50%, 2 if sten-

oses involved 50 to 99% reduction and 3 if total occlusion was present. The sum of the points was called the angiographic score.

Results: The patients had significantly higher concentrations of CRP, median (interquartile range): 3.70 (1.78-7.40) *vs*. 1.40 (0.60-2.43), P < 0,001. The serum PAF-AH activity did not differ between patients and control group 405 (330-471) *vs*. 406 (359-479), P = 0.591. CRP concentrations and PAF-AH activity in patients with PAD was not significantly associated with the angiographic score (r = 0.07, P = 0.461; r = -0.08, P = 0.450, respectively). **Conclusions:** This study confirmed the role a low CRP concentrations as risk factors in the development of PAD, but there is no evidence that CRP concentrations or PAF-AH activity are related with the angiographic score.

P02-14

Differences in phenotyping heart failure patients- hsTroponin I versus hsTroponin T

Farinha R (1), Martins S (1), Silva N (2), Frioes F (3), Bettencourt P (3), Guimaraes J (2)

(1) Sao Joao Hospital, Clinical Pathology Department, Porto, Portugal
(2) Faculty of Medicine, University of Porto & Sao Joao Hospital, Biochemistry Department & Clinical Pathology Department, Porto, Portugal
(3) Faculty of Medicine, University of Porto & Sao Joao Hospital, Internal Medicine Department, Porto, Portugal

Corresponding author: jtguimar@med.up.pt

Background: The availability of high-sensitivity (hs) cardiac troponin (cTn) assays has allowed the detection of circulating cTn in patients with heart failure (HF) and without acute coronary syndrome. Some studies suggest that circulating cTn may define an intermediate phenotype in HF development. Our aim was to measure hscTnT and hscTnI in HF patients and compare the way these two different forms of cTn perform in classifying those patients.

Material and methods: Blood samples were collected from 165 HF patients (mean age of 74 ± 12 years, 79 males and 86 females) on admission and on discharge of the hospital. In both moments the concentration of hscTnI and hscTnT were measured (hscTnI by Abbott Architect® using CMIA; hscTnT by Roche Cobas® using ECLIA).

Results: The correlation coefficient was 0.876. The values for the 25%, 50%, 75% and the 95% percentiles were, for hsTnI (in ng/mL) 0.015, 0.033, 0.080 and 0.625, and for hsTnT (in pg/mL) 24.5, 39.9, 70.6 and 232.0, respectively. Observed agreement between quartis for both determinations was 52% (168), with 127 (39%) changing one quartile, 26 (8%) changing two quartiles and 4 (1%) changing three quartiles. Considering a cut-off value for acute myocardial infarction of 0.30 ng/mL for hscTnI and of 100 pg/mL for hscTnT we found (N = 325) agreement in 299 (92.0%). Differences were found in 26: 3 (0.9%) with hscTnI >0.30 ng/mL and 23 (7.1%) with hscTnT >100 pg/mL.

Conclusion: Measuring cTn with different methods will lead to disagreement in HF patients' classification.

P02-15

Serum superoxide dismutase activity: A potential biomarker of atherogenesis

Mujagic R, Crnokrak S, Županić D

General Hospital Pula, Clinical laboratory, Pula, Croatia

Corresponding author: renat.mujagic@pu.t-com.hr

Aim: The aim of this study is to assess association of nutritional status with serum copper, zinc-superoxide dismutase (SOD) and related atherosclerosis risk in light of "Reverse epidemiology" hypothesis in undernourished hemodialysed patients.

Materials and methods: Seventy male hemodialysed patients were enrolled in this clinical trial. Patients were divided into four subgroups according to the body mass index quartile ranges. Serum copper, zinc- superoxide dismutase activity was measured by commercial test reagent kit and reference materials; copper and zinc concentration

were measured by inductively coupled plasma mass spectrometry; and ceruloplasmin activity was spectrophotometricaly measured by standard method. Analysis of variance (ANOVA) and appropriate *post hoc* analyses were performed by Statistica version 7.0 software.

Results: High SOD activity and copper-zinc ratio level in undernourished hemodialysed patients (ANOVA, $P < 0.001$) were found. Thereafter, non-high-density lipoprotein-cholesterol (non-HDL-C) and triglyceride concentration were lower in undernourished hemodialysed patients (ANOVA, $P < 0.001$). Macrophages, especially foam cells, and endothelial cells in atherosclerosis lesion produce high amount of SOD that can be expelled in the blood. SOD activity increase can be result of atherosclerosis progression.

Conclusion: These findings are consistent with "Reverse epidemiology" that explain higher atherosclerosis risk in hypocholesterolemic undernourished hemodialysed patients. Hypocholesterolemia can be atherosclerosis risk factor in hemodialysed patients according to SOD activity increase with concomitant non-HDL-C concentration decrease. Copper and zinc status disbalance (e.g. copper-zinc ratio increase) or hydrogen peroxide production increase by SOD in undernourished patient can cause oxidative stress which may contribute to acceleration of atherosclerosis.

P02-16

Serum neuron-specific enolase and procalcitonin as predictors of survival in comatose cardiac-arrest

Illana F, Castillo C, Fuentes M, Ortega M, Arroyo M

Hospital Clinico San Carlos, Laboratory Medicine Department, Madrid, Spain

Corresponding author: fran_illana@hotmail.com

Background: Early prediction of neurological damage after cardiopulmonary resuscitation is a challenge to intensive care physicians. Serum neuron-specific enolase (NSE) and procalcitonin (PCT) are two biochemical markers used to predict poor outcome in this patients. The aim of this study is to establish the prognostic value of NSE and PCT in survival comatose patients after cardiopulmonary resuscitation.

Material and methods: A retrospective study was conducted in 58 patients (age: 62.7 ± 14.7 years) who suffered a cardiac arrest. Serum NSE and PCT were measured within three days after cardiac arrest using an enzyme inmunoassay in Kriptor. Statistical analysis was performed using SPSS-15. Mann-Whitney test and Receiver Operator Characteristics (ROC) curves were used to compare groups and determine the best cut-off for NSE and PCT to predict exitus. Logistic regression was used to compare ROC curves.

Results: The mortality was 53%. The median and IQR levels of NSE and PCT (NSE: 52.1 ng/mL; [36.9-105.9]; PCT: 3.47 ng/mL; [1.7-7.7]) in patients who died were higher than survivors (NSE: 23.1 ng/mL; [15.9-33.9]; PCT: 1.13 ng/mL; [0.2-4.7]) statistically significant ($P < 0.001$). The ROC curve showed a NSE cut-off of 35 ng/mL to predict a fatal outcome. Both specificity and sensitivity were 80%. PCT cut-off of 2.77 ng/mL showed a 71% of sensitivity and a 66% of specificity. However, no additional improvement in prediction of death was found with PCT measures.

Conclusion: Serum NSE is a helpful biochemical marker to predict neurological damage. Serum PCT concentrations did not provide additional information to assess neurological damage in comatose patients after cardiac arrest.

P03 – Clinical chemistry 1

P03-01

The importance of the clinical state for successful collection of sweat sample in children

Matica J, Aralica M

Clinical Hospital Centre Rijeka, Clinical Department of Laboratory Diagnostics, Rijeka, Croatia

Corresponding author: jasminka.matica@ri.t-com.hr

Background: The sweat test is the "gold standard" for diagnosis of cystic fibrosis. Patient's physiological and clinical states are important factors for successful collection of sufficient quantity of sweat after pilocarpine iontophoresis. Aim of the study was to investigate clinical state of children regarding successful sweat collection.

Materials and methods: The questionnaire composed of seven questions was presented to parents or nurses accompanying tested children prior the sweat testing. The questions were shaped as statements describing the clinical state of the child (dehydration, fasting, elevated body temperature, acute illness, edema and skin lesions). Regarding presence or absence of listed clinical symptoms answers were considered as favorable (1 point) or unfavorable (0 points). A total sum (clinical state score, ranged 0-7) was introduced as measure of clinical state. The participants were divided into two groups according to the quantity of collected sweat (sufficient and non-sufficient). Results were presented as median (range) and Mann-Whitney test was used to calculate the difference in clinical state score between groups.

Results: There were 117 participants; 90 children (age 30 (1-196) months) in sufficient sweat quantity (SSQ) group and 27 (age 25 (1-208) months) in non-sufficient sweat quantity (NSSQ) group. The SSQ group had clinical state score 5 (3-7), and NSSQ group had 5 (3-6) but there was significant difference in clinical state score between groups (P = 0.006).

Conclusion: Study pointed out the importance of favourable clinical status of children undergoing sweat testing. Preanalytical phase regarding proper patient's preparation is crucial for successful collecting of the sweat.

P03-02

Relationship between gamma-glutamyltransferase levels with alcohol consumption in a young population

Abreu R, Leitão M, Almeida A, Bellém F, Cardoso V, Pinto A

Escola Superior de Tecnologia da Saúde de Lisboa (ESTeSL), Clinical Analyses and Public health, Lisbon, Portugal

Corresponding author: mcleitao@estesl.ipl.pt

Background: Excessive alcohol consumption is a worldwide increasingly problem, particularly in young people. The present study aimed to evaluate changes of gamma-glutamyltransferase (YGT) activity, caused by alcohol, in a young population.

Materials and methods: The sample consisted of 149 individuals, 108 females and 41 males, aged between 18 and 25 years. We used a questionnaire to collect personal data and socio-economic habits of alcohol. It was also collected blood samples by venipuncture to determine YGT activity. The study focused on the variables "age", "sex", "YGT activity value," and "level of alcohol consumption". Statistical analysis included descriptive and inferential methodologies.

Results and conclusions: Gender mean values of YGT activity were significantly different, being of 23.03 U / L in men and 16 U / L in women. For "level of alcohol consumption" the mean values of YGT were 16.49 U / L in abstinent, 18.10 U / L in moderate drinkers and 22.12 U / L in abusive consumers, but with differences not statistically significant. We concluded that alcohol consumption didn't change significantly YGT activity values in the studied sample. Shall be considered in further studies, the inclusion of other factors, which may be associated with liver damage from alcohol, as obesity or genetic predisposition.

P03-03

Prealbumin: nutritional marker in intensive care patients?

Cachapuz I (1), Lobo L (2), Noronha M (2), Lopes E (1), Amaro A (3), Granja C (3), Alves V (1)

(1) Pedro Hispano Hospital; Matosinhos Local Health Care Unit, Clinical Pathology Service, Oporto, Portugal
(2) Pedro Hispano Hospital; Matosinhos Local Health Care Unit, Nutrition Service, Oporto, Portugal
(3) Pedro Hispano Hospital; Matosinhos Local Health Care Unit, Intensive Care Medicine Service, Oporto, Portugal

Corresponding author: cachapuz.isabel@gmail.com

Background: The prevalence of malnutrition in critically ill patients lies around 40%. Due to the acute severe disease, it is particularly difficult to assess these patients' nutritional state. The aim of this study was to evaluate the validity of prealbumin (PA) as a nutritional marker in intensive care patients.

Material and methods: Exploratory study in 46 patients of the Intensive Care Unit (ICU) from the Unidade Local de Saúde de Matosinhos. For each patient PA (immunoturbidimetry) and nitrogen balance (NB) were calculated. We studied the association of these variables with: (1) severity indicator (APACHE II: Acute Physiology and Chronic Health Disease and SAPS II: Simplified Acute Physiology Score II), (2) length of stay and (3) ICU mortality. Statistical analysis was performed with SPSS (vs 18). **Results**: From the 46 patients (M:33/F:13), 24% died in the ICU. SAPSII and APACHE indexes present a statistically significant correlation ($r = 0.819$; $P < 0.01$]. PA is not correlated to NB but it is positively correlated to absolute NB ($r = 0.269$; $P < 0.05$). PA has no statistically significant correlations with length of stay and severity indexes. The T test does not reveal statistically significant difference in PA and NB values when the patient was discharged or died in the ICU.

Conclusions: Results reveal no statistical evidence for associating PA with NB. According to some bibliographic references, acute inflammatory processes can interfere in prealbumin results. Further studies are required to confirm the usefulness of PA as a biochemical marker for the evaluation of the nutritional state in critically ill patients.

P03-04

Plasma homocysteine and cerebrospinal fluid biomarkers in patients with Alzheimer's disease

Petek Tarnik I (1), Katušić Hećmović S (2), Malnar M (2), Trkanjec Z (3), Bukovec Megla Ž (1)

(1) University Hospital Centre "Sestre Milosrdnice", Laboratory of Endocrinology, Department of Oncology and Nuclear Medicine, Zagreb, Croatia
(2) Ruđer Bošković Institute, Division of Molecular Medicine, Zagreb, Croatia
(3) University Hospital Centre "Sestre Milosrdnice", Department of Neurology, Zagreb, Croatia

Corresponding author: iva.petek.tarnik@kbcsm.hr

Background: Alzheimer's disease (AD) is progressive neurodegenerative disorder characterized by formation of senile plaque and neurofibrillary tangles in the brain. There are no adequate therapies and no tests that would enable accurate diagnosis of AD. In this pilot study we investigated the role of plasma homocysteine levels as vascular risk factor and levels of proteins Aβ42, total-tau and p181-tau in cerebrospinal fluid (CSF) in development of AD.

Materials and methods: We analyzed plasma and CSF samples of clinically assesed AD individuals ($N = 9$, mean age 73 years), healthy age-matched individuals ($N = 8$, mean age 63 years) and demented patients with no certain diagnosis ($N = 8$, mean age 69 years). Plasma homocysteine levels were determined by HPLC-FD method. The CSF levels of Aβ42, total-tau and p181-tau were determined using ELISA assay, and total-tau/Aβ42 and p181-tau/Aβ42 ratios were calculated.

Results: We found high levels of plasma homocysteine in group with AD, so levels of homocysteine differed significantly between non-demented and AD group. We also identified that low Aβ42 and high total-tau or high p181-tau levels are characteristic CSF profile for probable Alzheimer's disease. All CSF biomarkers and their ratios differed significantly between non-demented and AD group and t-tau/Aβ42 ratio suggested that these biomarkers may be used for differential and early diagnosis of probable Alzheimer's disease.

Conclusions: Our pilot study supports CSF proteins and t-tau/Aβ42 levels as early biomarkers of Alzheimer's disease, also suggesting that homocysteine is a significant vascular risk factor for AD.

P03-05

One-step whole blood fluorometric emission scan method for diagnosis of cutaneous porphyrias

Kurt I, Ozturk O, Sertoglu E, Tapan S, El-Fawaer S, Bilgi C

Gulhane School of Medicine, Department of Medical Biochemistry, Porphyrin Unit, Ankara, Turkey

Corresponding author: erdimsertoglu@gmail.com

Porphyrias are caused by enzyme defects in hem biosynthesis leading to overproduction of porphyrins and their precursor. Although, spectrofluorometric screening of plasma is useful in symptomatic phase of cutaneous porphyrias, it is insufficient in cases with increased erythrocyte protoporphyrin. We developed a simple fluorometric method, based on Lamola (1975), which determine zinc and free protoporphyrin in whole blood. Fluorometric measurements were performed with Perkin-Elmer LS55 spectrofluorometer. Zinc protoporphyrin were obtained from Frontier Scientific. EDTA-whole blood was used as sample. In fluorometric measurement, fluorescence emission peaks' height at 594 nm ve 625 nm were assessed by taking direct fluorescence intensity after Allen correction. It has been approved that emission peaks at 594 and 625 nm belong to ZPP and free protoporfirine (FEP), respectively by adding ZPP and FEP sequentially. In practice, emission scan was performed between 500-700 nm wavelength at 428 nm exitation wavelength in fluorometer from 1/400 diluted hemolysate, which was prepared with 0.1 M Tris-HCl buffer (pH = 8.0) containing 0.2 % tween-20. The height of the peak at 594 nm, which belongs to ZPP, was compared with calibration graph and results were expressed as ZPP µmol/L and ZPP/hem (µmol/mol). The intra-day and between days coefficient of variations were 3.3% and 3.6 % respectively. Reference range of 125 healthy individuals between 20-72 years of age was found 16.0-57.0 µmol/mol. Whole blood fluorescence emission scan allows semi-quantitative assessment of whole blood erythrocyte protoporphyrin levels. It can be used initial test with plasma fluorescence emission scan for the diagnosis of cutaneous porphyrias.

P03-06

Impact of inflammation on high density lipoprotein function in polycystic ovary patient

Gidwani S (1), Phelan N (2), Mcgowan A (2), Gibney J (2), Young I (3), McEneny J (3)

(1) Royal Victoria Hospital, Belfast Trust, Clinical Biochemistry, Belfast, United Kingdom
(2) Adelaide and Meath Hospital, Department of Diabetes and Endocrinology, Dublin, Ireland
(3) Queens University Belfast, Centre for Public Health, Belfast, United Kingdom

Corresponding author: gidwanianand@hotmail.com

Background: Polycystic ovarian syndrome (PCOS) is a common endocrine disorder. It is associated with decreased high density lipoprotein (HDL) levels and low grade inflammation, which both contribute to atherosclerosis development. HDL particles undergo continual remodelling, which can be influenced by inflammation and affect HDL function. This study aims to evaluate the impact of inflammation on HDL function in PCOS.

Materials and methods: PCOS subjects (N = 100) were matched for BMI and percent body fat with control subjects (N = 100). HDL was subfractionated into HDL2 and 3 by rapid ultracentrifugation. The inflammatory molecule serum amyloid A (SAA) was measured in serum and HDL2 and 3 by an ELISA procedure. The protein concentration of HDL2 and 3 was measured by a colorimetric assay. The activities of phospholipid transfer protein (PLTP) and cholesterol ester transfer protein (CETP)

in HDL2 and 3 were measured by fluorimetric assays.

Results: Compared to the control subjects, SAA was increased in serum and HDL2 and 3 in the PCOS subjects (serum, 30952 ± 22003 *vs*. 25051 ± 18353 µg/L; HDL2, 6.57 ± 4.04 *vs*. 5.13 ± 3.6 µg/mg protein; HDL3, 3.64 ± 2.53 *vs*. 2.60 ± 2.2 µg/mg protein; $P < 0.05$ for all comparisons). Furthermore, PLTP activity was increased in HDL3 in PCOS subjects (HDL3, 7.84 ± 2.5 *vs*. 6.90 ± 1.74 nmol/mg protein; $P < 0.05$). No difference was noted in the activity of PLTP in HDL2 or CETP in HDL2 and 3.

Conclusions: This is the first study to show that PCOS, independent of obesity, lead to inflammatory changes within HDL2 and 3 and functional changes in HDL3-PLTP. Overall, these changes would influence the remodelling and function of HDL, which would decrease their antiatherogenic properties and enhance atherosclerosis development/progression.

P03-07

Optimum cut-off value of salivary melatonin to discriminate between ADHD children and a control group

Gorriz-Pintado S (1), Pitarch-Castellano I (2), Orts-Costa JA (1), Puertas-Cuesta FJ (3)

(1) Hospital Universitario de la Ribera, Análisis Clínicos, Alzira (Valencia), Spain
(2) Hospital Universitario de la Ribera, Pediatría, Alzira (Valencia), Spain
(3) Hospital Universitario de la Ribera, Neurofisología, Alzira (Valencia), Spain

Corresponding author: sigorriz@hospital-ribera.com

Background: Altered patterns of melatonin circadian rhythm have been described in sleep disturbances, neurological, psychiatric and immunological conditions. Attention Deficit Hyperactivity Disorder (ADHD) is the most commonly psychiatric disorder in children. The aim of this study is to determine if there is an alteration of the melatonin secretion pattern in ADHD children and therefore if it can be helpful for its diagnostic.

Materials and methods: Salivary samples, timed at 180 minutes before and 60 minutes after going to sleep, were collected from 46 healthy and 91 ADHD children. Quantitative determination of melatonin was performed by a direct non-extraction ELISA assay using DSX analyzer. Data were analyzed by ROC curves using SPSS 15.0 statistical analysis package.

Results: Melatonin mean values found at -180 and + 60 for the healthy and ADHD groups were: 1.8, 41.0; and 4.3, 27.3 pg/mL, respectively. In the healthy group, Dim Light Melatonin Onset increase (Δ DMLO) was 39.2 ± 24.4 (SD) pg/mL, 1.7 times higher than in the diagnosed group, 23.0 ± 15.9 (SD) pg/mL. The area under the ROC curve found was 0.71 (95%:0.612-0.806).Taking into account several cut-offs, we have considered 28 pg/mL as the optimum value, with 70% specificity and 63% sensitivity.

Conclusion: Δ DLMO values lesser or equal to the cut-off value may be suggestive of ADHD since this condition is related with a deficit in the secretion of melatonin. Therefore, Δ DLMO could be helpful in the diagnosis of ADHD and would be considered in the decision-making process.

P03-08

Dim Light Melatonin Onset assessed with only two timed salivary samples

Orts-Costa J (1), Pitarch-Castellano I (2), Tárrega-Roig E (1), Puertas-Cuesta F (3)

(1) Hospital Universitario de la Ribera, Análisis Clínicos, Alzira (Valencia), Spain
(2) Hospital Universitario de la Ribera, Pediatría, Alzira (Valencia), Spain
(3) Hospital Universitario de la Ribera, Neurofisología, Alzira (Valencia), Spain

Corresponding author: jaorts@hospital-ribera.com

Background: Dim Light Melatonin Onset (DLMO) is the single most accurate marker for assessing

the circadian pacemaker. The salivary melatonin measurement is a reliable alternative to blood samplings, being particularly suitable for children because it offers a non-invasive and stress-free collection. Altered patterns of melatonin circadian rhythm have been described not only in sleep disturbances but also in neurological conditions. We assessed if taking only two timed samples is enough to show an alteration in the DMLO pattern. For this purpose, we have determined DLMO in healthy and affected by Attention Deficit Hyperactivity Disorder (ADHD) children.

Materials and methods: Salivary samples, timed at: 3 (-3), 2 (-2) hours before, during (0), and 1 hour after (+1) going to sleep, were collected from 46 healthy children and from 91 ADHD children diagnosed. Specific instructions for specimen collection were given. Suitable samples were centrifuged 10 min to remove particulate material and frozen at -70 ºC until analysis. Quantitative determination of melatonin in saliva was performed by a direct non-extraction ELISA assay on a DSX analyzer. Statistical analysis was performed by SPPS 15.0.

Results: Mean values at different times expressed as pg/mL were, in healthy children: 1.9, 3.0, 9.0, 27.2 and 41.0; in ADHD children were: 4.3, 5.1, 11.7, 20.8, 27.1; both in 3,2,1,0, and +1 hours, respectively. The greatest difference among different timed samples was found between -3 and +1 hours.

Conclusion: DLMO pattern assessment could be simplified by considering only two timed samples at -3 and +1 hours, making easier in this way the sampling taking process.

P03-09

Thrombocyte serotonin and serum cholesterol concentration in depressed patients

Ruljancic N (1), Mihanovic M (2), Cepelak I (3)

(1) Psychiatric Hospital "Sveti Ivan", Department of Laboratory Diagnostics, Zagreb, Croatia
(2) Psychiatric Hospital "Sveti Ivan", Department of Psychiatry, Zagreb, Croatia
(3) Pharmaceutical-Biochemical Faculty, University of Zagreb, Department of Medical Biochemistry and Hematology, Zagreb, Croatia

Corresponding author: n_ruljancic@yahoo.com

Introduction: Numerous studies have confirmed the connection of reduced serum cholesterol and thrombocyte serotonin concentration with suicidal behaviour in psychiatric patients.

Materials and methods: In accordance to ICD-10, 55 depressed patients with suicide attempt and 77 depressed patients with no suicide attempt were separated in two subgroups; F32.2 and F33.2. Control group was presented by the healthy blood donors. The fasting serum cholesterol concentration was established using standard enzymatic method, while the thrombocyte serotonin concentration was determined by ELISA method.

Results: The ANOVA test ($N = 228$, $F = 8.26$, $P < 0.001$) found significant difference of cholesterol concentration, with lowest concentration in the group of depressed patients with attempted suicide, diagnoses F32.2 ($P = 0.031$) and F33.2 ($P = 0.011$), compared to the group of depressed patients without attempted suicides. Upon gender stratification, the significance remained for the female patients (ANOVA, $N = 125$, $F = 6.06$, $P = 0.003$). The thrombocyte serotonin was found to be significantly different in all groups (SNK *post hoc* test, $P < 0.05$, $N = 187$, $F = 37.69$, $P < 0.001$), with the lowest thrombocyte serotonin in the group of depressed patients with no suicide attempt (F32.2), compared to the same diagnosis with suicide attempt (MW-test, $P = 0.018$). The same significance was found for the group of female (ANOVA, N =

103, F = 11.81, P < 0.001) and male patients (ANOVA, N = 84, F = 30.40, P < 0.001).

Conclusion: In the group of depressed patients with attempted suicide, lower serum cholesterol values have been confirmed. In the group of depressed patients with no suicide attempt, lower values of thrombocyte serotonin have been confirmed, presumably as the response to the psychopharmacological therapy.

P03-10

Effect of melatonin administration on lipid peroxide and antioxidant levels in tissues of SHR

Kitagawa A (1), Ohta Y (2)

(1) Shigakkan University, Department of Nutrition, Faculty of Wellness, Ohbu, Japan
(2) Fujita Health University, Department of Chemistry, School of Medicine, Toyoake, Japan

Corresponding author: kitagawa@fujita-hu.ac.jp

Background: Spontaneously hypertensive rats (SHR) show the symptom of metabolic syndrome and hypertension. Melatonin (MT) with antioxidant properties is known to inhibit hypertension in SHR. However, there are few reports on the antioxidant effect of MT in SHR. Therefore, we examined the effect of MT administration on lipid peroxide and antioxidant levels in the liver, kidney and heart of SHR.

Material and methods: Five-week-old male SHR and Kyoto Wistar rats (WKY) were fed MF diet. MT (1 or 10 mg/kg body weight) was orally administered to SHR once every morning for two weeks, starting at four-week feeding. Systolic blood pressure (SBP) was measured before sacrifice. Livers, kidneys, and hearts were used for measurements of lipid peroxide (LPO), ascorbic acid (AA), and reduced glutathione (GSH).

Results and conclusion: Hepatic, renal, and cardiac LPO contents and SBP were higher in SHR than in WKY. MT administration reduced these LPO contents and SBP in SHR. There were no differences in hepatic, renal, and cardiac GSH contents between SHR and WKY. MT administration did not affect these tissue GSH contents in SHR. Hepatic and cardiac AA contents were higher in SHR than in WKY. Renal AA content was less in SHR than in WKY. MT administered to SHR elevated the liver and renal AA contents and reduced the cardiac AA content. These results indicate that MT administered to SHR attenuates increased blood pressure and hepatic, renal, and cardiac LPO contents, although the administered MT has different effects on the changes in tissue AA contents.

P03-11

Adipocyte fatty acid binding protein and cardiovascular risk in women with overweight/obesity

Sypniewska G (1), Mankowska-Cyl A (1), Bergmann K (1), Rajewski P (2)

(1) Collegium Medicum Nicolaus Copernicus University, Dept. of Laboratory Medicine, Bydgoszcz, Poland
(2) E. Warminski City Hospital, Department of Internal Diseases, Bydgoszcz, Poland

Corresponding author: grazynaodes@interia.pl

Background: Fatty acid-binding protein (A-FABP) derived from adipocytes and macrophages has been suggested a biomarker for obesity and metabolic syndrome. We evaluated the association of A-FABP with proatherogenic profile and insulin resistance in young non-diabetic overweight/obese women.

Materials and methods: A-FABP, hsCRP, adiponectin, lipids and apolipoproteins were measured in blood samples obtained from 104 women aged 25-40 yrs, BMI ≥ 25 kg/m^2 and age-matched healthy controls (N = 76; BMI < 25kg/m^2).

Results: In overweight/obese women (BMI 32.6 ± 6.1 kg/m^2) serum A-FABP correlated positively and more strongly with hsCRP (P < 0.001) than with anti-inflammatory indicators like adiponectin, HDL-C, ApoA1 (negative correlation). A-FABP was associ-

ated with BMI, HOMA-IR, insulin ($P < 0.003$) and atherogenic profile indices, especially ApoB/ApoA1, TC/HDL-C, TG/HDL-C ($P < 0.003$). In the study group A-FABP, but neither adiponectin nor BMI, was an independent predictor of ApoB ($\beta = 0.96$; $P = 0.03$), ApoA1 ($\beta = 0.68$; $P = 0.02$), ApoB/ApoA1 ($\beta = 1.21$; $P = 0.01$) and TG/HDL-C ($\beta = 0.71$; $P = 0.004$). A-FABP compared to adiponectin was of very good diagnostic accuracy for discrimination of women with atherogenic risk profile and insulin resistance (CRP ≥ 1 mg/L; TG/HDL-C ≥ 3). AUC for A-FABP and adiponectin were 0.80 *vs*. 0.59 ($P < 0.003$) and 0.88 *vs*. 0.73 ($P < 0.08$), respectively. A-FABP compared to adiponectin was of excellent accuracy (AUC = 0.96 *vs*. 0.67; $P < 0.001$) at a cut-off value 16 ng/mL for discrimination of women with increased BMI and atherogenic risk profile with an odds ratio of 11.2 (95% CI 3.7-34.2), 7.1 (1.9-27.2), 6.7 (2.6-17.2) for having elevated TG/HDL-C, apoB, CRP.

Conclusions: In young non-diabetic overweight/obese women serum A-FABP seems to be a very good predictor of atherogenic risk profile and insulin resistance.

P03-12

Determining the concentration of the hormone leptin and ghrelin in the treatment of obese people

Posavec L (1), Krpan R (1), Nikolić M (2), Miroševi ć G (3), Bukovec Megla Ž (1), Stančić V (4)

(1) University Hospital Centre "Sestre milosrdnice", Laboratory of Endocrinology, Department of Oncology and Nuclear Medicine, Zagreb, Croatia
(2) University Hospital Centre "Sestre milosrdnice", Division of Gastroenterology and Hepatology, Department of Internal Medicine, Zagreb, Croatia
(3) University Hospital Centre "Sestre milosrdnice", Division of Endocrinology, Diabetes and Metabolic Diseases, Department of Internal Medicine, Zagreb, Croatia
(4) University Hospital Centre "Sestre milosrdnice", Division of Hematology, Department of Internal Medicine, Zagreb, Croatia

Corresponding author: ljubica.posavec@kbcsm.hr

Background: Obesity is the excessive accumulation of adipose tissue in the body, and is expressed as body mass index (BMI). By definition of the World Health Organization, a BMI ≥ 30 kg/m^2 is considered to be obesity. Objective of this study was to examine the concentration changes of hormones leptin and ghrelin before and after six months of treatment of obesity, depending on the body weight loss and grade of obesity.

Materials and methods: The study enrolled 44 patients treated with intragastric balloon, and 36 operated patients. Leptin concentrations, the so-called satiety hormone, were measured by EIA method (Biosource) and ghrelin concentrations, the so-called hunger hormone, were measured by RIA method (Biosource).

Results: Patients treated with intragastric balloon had higher initial concentrations of ghrelin (median 954 pg/mL) in comparison to surgical patients (median 777 pg/ml); $P < 0.001$, as expected given their initial body weight (114 to 128.5 kg). Initial leptin concentrations of patients with intragastric balloon were also higher (median 26.9 ng/mL) compared to surgical patients (median 15.2 ng/mL); $P < 0.001$. However, lower concentrations of leptin in surgical patients results from previously induced weight loss.

Conclusions: The concentration of leptin decreases with weight loss, regardless of the method of treating obesity, whereas ghrelin is negatively correlated with body weight. The concentrations of the hormone leptin and ghrelin through six months of treatment are statistically significantly different. Higher initial values of ghrelin indicate to greater weight loss of patients treated with "sleeve" resection of the stomach and gastric band.

P03-13

Elevation of urinary porphyrins after prolonged sedation with Propofol. Fact or interference?

Castillo Perez C (1), Illana Camara F (1), Torrejon M (1), Velasco G (2), Ferrero J (2), Arroyo M (1)

(1) Hospital Clinico San Carlos, Laboratory Medicine, Madrid, Spain
(2) Hospital Clinico San Carlos, Cardiovascular Intensive Care Unit, Madrid, Spain

Corresponding author: carloscp2@hotmail.com

Background: Porphyries are a group of metabolic diseases caused by the deficiency of an enzyme involved in heme synthesis. Certain drugs may precipitate a porphyric crisis. One anesthetic considered safe for using in these patients is Propofol. However, porphyrin elevation has been described after a prolonged sedation with Propofol in some studies.

Material and methods: We measured 24 hours urinary porphyrin excretion in 4 patients sedated with Propofol for at least 24 hours and after Propofol withdrawal. The method used for the porphyrin determination was reverse phase HPLC with fluorescence detection and a column C18 as stationary phase. Interference studies were performed using β-glucuronidase and sulfatase type H-1 enzymes.

Results: An increase in total urinary porphyrin excretion as well as an increase in hexaporphyrin and uroporphyrin was found after Propofol sedation. Progressive normalization of urinary porphyrins was found after anesthetic withdrawal. No differences were found after using of glucuronidase and sulfatase enzymes.

Conclusions: Prolonged Propofol sedation caused an increase in the total urinary porphyrins, uroporphyrin and hexaporphyrin and it does not seem to be mediated by urinary propofol excretion metabolites. More studies are needed to elucidate the origin of this increase.

P03-14

Glutathione peroxidase and reduced glutathione levels depend on aging

Cinar E, Bilgin R, Yalcin SM

University of Cukurova, Arts & Science Faculty, Chemistry Department (Biochemistry Division), Adana, Turkey

Corresponding author: rbilgin@cu.edu.tr

Free oxygen radicals have been proposed as important causative agents of aging. Aging is characterized by a progressive change in biochemical an functions in various tissues and organs in an individual. Clear reasons for this decline is unkown, but it has been suggested that increased oxidative stress, energy metabolism, immune system can take an important role. Free radical theory of aging predicts and its modern version, the oxidative stress theory of aging, the production of reactive oxygen species with increasing age and antioxidant stress deterioration of the delicate balance between antioxdant system may lead to a change in the cellular environment In this study, we determined erythrocyte glutathıone peroxidase and reduced glutathione levels to evaluate the effect of aging in healthy subjects of different ages. For this purpose, 84 healthy subjects divided in four groups as 2-12; 13-24; 25-40; 41-69 of age were investigated. Glutathione peroxidase levels were not found statistically significant in among the all age groups. On the other hand, reduced glutathione levels were found statistically significant difference in these age groups 13-24 and 25-41 ($P < 0.05$).

P03-15

Acute intermittent porphyria induced by valproate: a clinical case.

Garcia Moreira V, Fernandez Leivas A, Garcia Alonso S, Martinez Gago M, Laborda Gonzalez B, Fernandez Rodriguez E

Hospital de Cabueñes, Servicio de Analisis Clinicos, Gijon, Spain

Corresponding author: eloyfr@gmail.com

Background: Acute intermittent porphyria (AIP) is an autosomal dominant inherited disease caused by a decreased activity of hydroxymethylbilane sintase (HMBS)/porphobilinogen deaminase (PBGD), an enzyme in the heme biosynthetic pathway. Clinically is characterized as acute neurovisceral attacks, often precipitated by exogenous factors such as drugs, hormones, and alcohol.

Case: A 46-year-old woman presented to our hospital with unprovoked complex partial seizures post-stroke. She was treated with levetiracetam/valproate/atorvastatin. A month later, she was admitted with psycomotor agitation. Levetiracetam was suspended. She continued with abdominal pain, nervousness, insomnia and generalized tremor. Most notable of the laboratory analysis was: ALT 55 U/L and AST 56 U/L. Other general determinations were normal. Porphyrins analysis were subsequently requested: ALA 75.5 mg/24h (0-7.5), PBG 54.25 mg/24h (0-2.5), total porphyrins 514.9 mcg/24h (0-150), mainly due to uroporphyrins 191.9 mcg/24h (0-25) and pentacarboxiporphyrins 26.6 mcg/24h (0-5). Fecal porphyrins were normal. Treatment with valproate was suspended. In the study of the erythrocyte HMBS activity, values were normal. The genetic study of HMBS gene revealed that the patient was a heterozygous carrier of a deletion in exon12:c.669-698del30(p. Glu223-Leu232del). The three children of the patient were genetically analyzed. Two were also carriers of the mutation. Patient was referred to a specialized porphyrias unit for monitoring and currently remains asymptomatic.

Conclusion: Valproate and most antiepileptic drugs are unsafe because they have demonstrated porphyrinogenicity. The detection of asymptomatic carriers of the mutation can help avoid and treat early crises, often underdiagnosed for their specificity clinic. As we see, although porphyria usually is not considered in the differential diagnosis of hypertransaminasemia, it could be a cause.

P04 – Clinical chemistry 2

P04-01

Evaluation of new point of care blood gas system Rapidpoint 500

Baršić I, Brkljacic V, Rogic D, Sertic J

University Hospital Center Zagreb, Clinical Institute of Laboratory Diagnostics, Zagreb, Croatia

Corresponding author: ibarsic@kbc-zagreb.hr

Background: The aim of the study was to evaluate the performance of a new Rapidpoint 500 blood gas system and assess its capability as a point of care analyzer. The results of pH, blood gases, electrolytes, glucose, lactate and total hemoglobin were compared with results obtained on Rapidlab 1265 blood gas analyzer, and neonatal bilirubin results were compared with results obtained on biochemistry analyzer Cobas 6000 series module c501.

Materials and methods: The evaluation was performed at the Department of Anesthesiology and Intensive Care Unit (pH, blood gases, electrolytes, glucose, lactate and total hemoglobin) and at the Department of Pediatrics, Division for Neonatology and Intensive Care (neonatal total bilirubin). We collected arterial blood with balance heparin (N = 52), and capillary and venous samples (N = 40) for neonatal bilirubin. Capillary and venous samples were collected by nurses at the Department of Pediatrics, so we were able to investigate the influence of non-laboratory staff on results obtained from the point-of-care device and in central laboratory. We calculated imprecision, inaccuracy and

comparability with Rapidlab 1265 and Cobas 6000 series module c501.

Results: Within-run imprecision CVs were all below 5% and the results showed acceptable inaccuracy for all analytes. The correlation with the comparative methods was satisfactory; correlation coefficients were between 0.9116-0.9938 for all parameters. Passing-Bablok regression analysis of analyte concentrations showed good compatibility.

Conclusion: Rapidpoint 500 showed good imprecision (CV < 5%), acceptable inaccuracy and comparability with central laboratory and it can be used as a point-of-care device in intensive care units and neonatology.

P04-02

Evaluation of the Enterprise Point of Care meter (EPOC) for the emergency ward

Buurman S (1), Heringhaus C (2), Schenk P (1), Cobbaert C (1)

(1) Leiden University Medical Center, Department of Clinical Chemistry, Leiden, Netherlands
(2) Leiden University Medical Center, Department of Emergency Medicine, Leiden, Netherlands

Corresponding author: c.m.cobbaert@lumc.nl

Background: Rapid availability of blood gas, metabolite and electrolyte results is crucial for decisions about further medical treatment of reanimation patients at the Emergency Ward. Therefore, we evaluated the Epocal Enterprise Point of Care meter (EPOC) versus the Siemens Rapidlab 865 analyzer intended for central laboratory blood gas measurements.

Materials and methods: Reproducibility of the EPOC parameters was tested using Eurotrol GASISE Metabolites reference material. Method comparison was performed using 30 freshly collected patient samples, which were applied to both devices within 10 seconds. Clinical equivalence was tested for nine parameters by 2-Instrument Comparison (EP Evaluator, Rhoads). An Error Index (the ratio between the (Y-X) difference and allowable total error) was calculated per parameter and per sample. Methods are clinically equivalent if the absolute value of (Y-X) is smaller than allowable error in at least 95% of samples.

Results: Overall reproducibility was < 2% for all parameters except pO2. EPOC pH and lactate measurements were clinically equivalent to those from the Rapidlab 865. However, EPOC and Rapidlab sodium, potassium, ionised calcium, glucose, pO2, pCO2, and haematocrit results were not clinically equivalent, with Error Indices < -1 or > 1 in 10-87% of samples.

Conclusions: The significance of POCT blood gas-electrolyte-metabolite analyses with short turnaround times for critical patients is well known. Whereas the evaluation of the EPOC meter shows excellent reproducibility for all parameters tested, there are clinically relevant differences versus central laboratory equipment for 7 out of 9 parameters. These data clearly illustrate the need for a quality mark for POCT devices.

P04-03

Performance of Nova StatStrip point of care blood glucose meter in a neonatal intensive care unit

Tendle K

Medical University of Vienna, Department of Pediatrics and Adolescent Medicine, Vienna, Austria

Corresponding author: d.dobson@novabiomedical.co.uk

Background: Monitoring blood glucose in neonatal intensive care (NICU) patients is important in maintaining normoglycaemia and reducing the risk of hypoglycaemia. POC glucose meters provide short turnaround times (TAT) but it has been reported that the accuracy of many commonly used meters can be affected by hematocrit interference and other biochemical or biological substances present in neonatal whole blood. The aim of this study was to challenge the performance of a POC glucose meter

(Nova Biomedical StatStrip) that corrects for interfering substances, to determine its clinical reliability in a challenging pre-term neonatal population.

Methods: StatStrip was tested on 159 heparinised whole blood neonatal samples obtained for blood gas analysis. Patients tested had varying levels of hematocrit, pH and pO2, and received a range of medication. The accuracy of Statstrip Glucose was evaluated by comparing the results of the meter with the results of the blood gas analyser ABL 700 (Radiometer Medical ApS) routinely used for glucose measurements in this NICU setting.

Results: StatStrip results correlated closely to the ABL 700 ($r^2 = 0.97456$) across a wide glucose concentration range including the hypoglycaemic range (min = 13 mg/dL; max = 389 mg/dL). The varying levels of hematocrit pH and pO2 present in the whole blood samples did not affect the accuracy of results. The accuracy of results was also unaffected by the medication used and other factors, e.g. birth weight or patient's age.

Conclusions: StatStrip showed good clinical accuracy and performance and is a suitable alternative to a blood gas analyser for measuring glucose in NICU patients.

P04-04

A large-scale randomized clinical trial on the effectiveness of reflective testing in primary care

Verboeket-van de Venne W, Kleinveld H, Oosterhuis W

Atrium Medical Centre, Department of Clinical Chemistry, Heerlen, Netherlands

Corresponding author: wvenne@atriummc.nl

Background: Reflective testing is a procedure in which the laboratory specialist adds additional tests and/or comments to an original request, after inspection (reflection) of the results. The aim of the present study is to evaluate the effects of reflective testing on patient treatment and outcome.

Materials and methods: We started with laboratory reports from 600 patients that were suitable to be subjected to reflective testing. After randomization, general practitioners (GPs) of 300 patients received the additional tests and comments (intervention). GPs of the other patients only received the results of the originally requested tests (control). After a follow-up period of six months, information was collected about following laboratory reports, treatments or referrals, other additional diagnostic procedures and specific patient data (e.g. medical history, medication).

Results: Informed consent to collect follow-up data was received from 350 patients. In 81 of them additional tests were performed without resulting in added value. Data of 269 patients (intervention: 148, control: 121) were further analyzed. Reflective testing was considered to be useful in 95% of the cases; 74% of the GPs (intervention) compared with 45% (control) had stated the intention of an adequate treatment action. An actual improvement of the care process was observed in 70% of the patients in the intervention group (vs. control: 46%; $P < 0.001$).

Conclusion: Reflective testing can be seen as a new dimension to the service of the clinical chemistry laboratory to primary health care. In our opinion, these results contribute significantly to the evidence of the effectiveness of reflective testing.

P04-05

Microdialysis-based biosensors in experimental and clinical research

Cibicek N (1), Vacek J (1), Hrbac J (2), Zivn H (3), Mandak J (4), Ulrichova J (1)

(1) Palacky University Olomouc, Faculty of Medicine and Dentistry, Department of Medical Chemistry and Biochemistry, Olomouc, Czech Republic
(2) Palacky University Olomouc, Faculty of Science, Department of Physical Chemistry, Olomouc, Czech Republic
(3) Charles University in Prague, Faculty of Medicine in Hradec Kralove, Radioisotope Laboratories and Vivarium, Hradec Kralove, Czech Republic
(4) Charles University in Prague, Faculty of Medicine and University Hospital in Hradec Kralove, Department of Cardiac Surgery, Hradec Kralove, Czech Republic

Corresponding author: norbert.cibicek@upol.cz

Background: Microdialysis, a sampling method originally developed for in situ experimental tissue metabolism monitoring, has also been utilized in (clinical) pharmacological / toxicological studies, for barrier function assessment and estimation of local blood perfusion.

Materials and methods: A contemporary literature review (years 2007-2012) was performed using Medline, Scopus and WOS databases with search terms "microdialysis" and "biosensor". Relevant studies were subgrouped into experimental and clinical research categories. The major methodological and application advances were summarized for both areas and compared with our own experimental and clinical experience.

Results: Concerning the experimental papers, neuroscience dominates the wide array of applications in various organs. In the area of clinical research, implantable microdialysis sensors are (still) focused on the brain, subcutaneous and intravenous measurements of glucose and its metabolites on one hand, and on the monitoring of neurotransmitters and pharmaceuticals on the other. In keeping with our practice, the major drawbacks of microdialysis biosensors remain in the realm of biointerface conditions, which affect their function and longevity. The technological evolution concentrates on increased sensitivity and lag-time reduction (employment of enzymes and on-line electrochemical detection mechanisms), continuous calibration techniques (e.g. with fluorescent probes), wireless setups and miniaturization (lab-on-a-chip).

Conclusions: In the experimental brain and diabetes research, microdialysis has become a well-established technique that may be used as a reference for the development of other (e.g. optical) biosensors. Clinical research attempts to identify the situations, where microdialysis could replace the standard methods. Acknowledgements This work was supported by grant project MPO FR-TI4/457.

P04-06

Pitfalls in measurement of lysosomal enzymes using dried blood spots on filter paper

Brzak M (1), Bilić K (2) Fumić K (2)

(1) Faculty of Pharmacy and Biochemistry, University of Zagreb, Zagreb, Croatia
(2) Department of Laboratory Diagnostics, University Hospital Center Zagreb, Zagreb, Croatia

Corresponding author: kbilic@kbc-zagreb.hr

Background: Increasing interest in dried blood spot (DBS) measurement of lysosomal enzymes is due to small sample volume and easy transport and storage. However, most pre-analytic procedures are not under laboratory control. The objective of this study was to analyze the effect of leukocytosis and the time period from blood drawing to spotting on filter paper for activity of α-galactosidase A and α-glucosidase.

Materials and methods: a) DBS samples from 130 individuals with and without leukocytosis who did not have the diagnosis of Pompe and Fabry disease; b) Aliquotes of 60 µl EDTA-blood from 65 healthy individuals were spotted on filter papers immediately after blood was drawn and then one hour after blood drawing without inverting the sample gently in the meantime. α-galactosidase A

and α-glucosidase activities were measured using in house fluorometric methods.

Results: The activities of α-galactosidase A (mean ± SD) and α-glucosidase (mean ± SD) were respectively (nmol/21h/mL): a) DBS samples with leukocytosis: 25.4 ± 17.09 and 28.7 ± 22.30; DBS samples without leukocytosis: 13.0 ± 11.00 and 20.0 ± 19.51; b) DBS prepared immediately after blood was drawn: 12.8 ± 10.89 and 19.9 ± 20.27; DBS prepared one hour after blood drawing without inverting the sample: 7.6 ± 7.42 and 7.2 ± 6.19.

Conclusions: DBS samples prepared during the period in which patients suffered from leukocytosis can lead to false negative results in juvenile or adult forms of Fabry or Pompe disease in patients with moderately lowered enzyme activity. Adequate use of DBS measurement requires continuous education of all participants in the diagnostic process.

P04-07

Clinical evaluation and etiologies of prolonged jaundice in IVF born neonates

Mehrotra V (1), Saini P (2)

(1) Himalayan Institute of Medical Sciences, Jollygrant, Clinical Biochemistry, Dehradun, India
(2) Saini Infertility Centre, Rajpur Road, Saini Infertility Centre, Dehradun, India

Corresponding author: mehrotravinit@rediffmail.com

Background: When jaundice persists beyond 14 days it is called prolonged or protracted neonatal icterus or jaundice. Etiology of this cause needs a pivotal step for management because a delay in the diagnosis and treatment may lead to serious complication or even death. The most common causes include hyperbilirubinemia (physiologic or hemolytic), breast feeding or breast milk jaundice. Considering all the possible etiologies as an essential a clinical evaluating is most crucial.

Material and methods: Hospitalized or outpatients of known IVF born neonates with prolonged jaundice were studied. General data of mother and neonates containing age, sex, weight, type of delivery, type of feeding, jaundice history and therapy was collected and clinical investigations: serum bilirubin (total and conjugate), liver functions tests, blood group, complete hemograme, thyroid function tests, glucose-6-phosphate dehydrogenase and urine culture were performed.

Results: One hundred IVF born neonates with prolonged jaundice were enrolled (male: 67 and female: 33) with the mean age of 21 days, weight 2.5 kg and jaundice onset was 5 days. 75% were breastfeeding and 7% shows urinary tract infection and 45% G6PD deficiency. 4% shows higher values of TSH or T4. Septicemia, Down syndrome and ABO incompatibility were present in only 1%. The neonate also shows good evidence of liver dysfunction or hypothyroidism. The etiological cause was unknown in 4% of the cases.

Conclusions: It was concluded that the most common clinical and etiologic causes of IVF born neonates with prolonged jaundice were breast feeding, G6PD deficiency, UTI and hypothyroidism correspondingly.

P04-08

Influence of the assay for measuring serum albumin on level of corrected calcium (CaC) concentration

Garcia Moreira V, Martinez Gago M, Fernandez Leivas A, Bachiller Sister M, Llorente Torres A, Fernandez Rodriguez E

Hospital de Cabueñes, Servicio de Analisis Clinicos, Gijon, Spain

Corresponding author: eloyfr@gmail.com

Background: The use of albumin-corrected calcium (CaC) is recommended as calcium measurement. Usually, albumin is measured by dye-binding assays: bromcresol green (AlbBCG) and bromcresol purple (AlbBCP) and systematic differences between them have been described. Our objective was to evaluate the impact of the albumin method on CaC concentrations.

Materials and methods: We measured calcium and albumin concentrations by BCG and BCP methods in 100 sera. CaC was calculated according to: CaC (mg/dL) = total Ca (mg/dL) +0.8 x [4-albumin (g/dL)]. Comparisons were performed using the Student t-test for paired data and the Bland-Altman plot, using the program MedCalc. Significance was set at $P < 0.05$.

Results: Total calcium mean was 8.81 mg/dL and standard deviation of 0.91. Mean concentration of AlbBCG was 36.81 ± 7.53 g/L and 30.96 ± 9.61 g/L for the AlbBCP. So, there were significant differences between methods, being 5.85 ± 3.28 g/L higher in AlbBCG is employed ($P < 0.01$). Mean CaC based on AlbBCG and AlbBCP was 9.36 ± 0.58 mg/dL and 9.82 ± 0.64 mg/dL, respectively ($P < 0.01$), with a mean difference BCG-BCP of -0.46. Using the AlbBCG to estimate CaC there was 1 "hypocalcaemic" (< 8.5 mg/dL), 12 "hypercalcaemic" (> 10 mg/dL) and 87 "normocalcemic" cases. Measuring AlbBCP theses cases were 1, 32 and 67, respectively. Thus, we have obtained a 20% discrepancy by using one or other method: 32 patients were classified as hypercalcemic when the AlbBCP was used, of which 20 would considered normal and 12 hypercalcemic using AlbBCG.

Conclusion: On average, AlbBCP are > 5g/L lower than AlbBCG. The choice of albumin method has a great impact on CaC calculation and therefore in the patients classification according to their phophocalcic status.

P04-09

Performance of the StatStrip POCT analyzer in detecting hypoglycaemic episodes in diabetic patients

Vučić Lovrenčić M (1), Radišić Biljak V (1), Božičević S (1), Car N (2)

(1) Merkur Teaching Hospital, Institute of Clinical Chemistry and Laboratory Medicine, Zagreb, Croatia
(2) Merkur Teaching Hospital, Vuk Vrhovac University Clinic, Zagreb, Croatia

Corresponding author: vucic@idb.hr

Background: Improving glycaemic control, whilst minimizing risk of hypoglycaemia is the ultimate goal of diabetes management. Intensified insulin treatment is the most common source of iatrogenic hypoglycaemia. Due to the hypoglycaemia unawareness, common in diabetes, many of the hypoglycaemic episodes remain undiagnosed and untreated, leading to a variety of adverse outcomes, ranging from mild autonomic symptoms to death. Accurate and readily available plasma glucose monitoring is of utmost importance in diagnosis and subsequent treatment of hypoglycaemia. The aim of this study was to evaluate the performance of a new type glucose sensor for the POCT glucose analyzer (StatStrip, Nova Biomedical, USA) in detecting hypoglycaemic episodes in diabetic patients upon institution of intensified insulin treatment.

Materials and methods: Capillary plasma glucose was measured by the StatStrip POCT glucose analyzer in hospitalized diabetic patients (N = 49) upon institution of intensified insulin treatment (glycaemic profile: 8 times per 24 hours) and results were compared to the reference hexokinase procedure (Olympus AU400, Beckman Coulter, USA).

Results: A total of 41 hypoglycaemic episodes (plasma glucose ≤ 3.9 mmol/L) were detected in the study period. There was no statistically significant bias between plasma glucose, as measured by the StatStrip and reference procedure (3.0 ± 0.83 *vs*. 3.1 ± 0.69 mmol/L, $P = 0.071$). Diagnostic test revealed a 95.1% sensitivity of the StatStrip in detecting hypoglycaemia, when tested against reference procedure.

Conclusions: The StatStrip POCT glucose analyzer showed excellent performance and clinical accuracy for detecting hypoglycaemic episodes in diabetic patients treated with intensified insulin therapy.

P04-10

Implementation of blood gas analysis POCT network in an Italian Health Organization with 3 hospitals

Bianchi V, Camurati C, Casalecchi M, Perticone V, Arfini C

SS Antonio e Biagio Hospital, Clinical Laboratory, Alessandria, Italy

Corresponding author: vbianchi@ospedale.al.it

Background: The debate on point of care testing (POCT) is an important aspect of Laboratory Medicine. Purpose of this work is to show a POCT network for blood gas analysis realized in Alessandria (Italy).

Materials and methods: Three hospitals far each other, one of these paediatric, with 11 critical wards. Project and implementation steps. First step: setting, needs analysis, study of connectivity. Second step: writing requirements, inviting tenders, carrying out task, appointing winner. Third step: instrument installation linked with a middleware server for data management. Fourth step: staff training, writing standard operation procedures, monitoring errors, managing warehouse, accounting tests.

Results: POCT network involves 11 analyzers and 11 wards. Both nurses and medical doctor can correctly instrumentations. A middleware server located in the Laboratory Unit collects all data and put them in electronic records. A total quality control is performed, when results are out the limit, POCT Technician switch off single electrodes or all instrumentation. Unique warehouse and stock control allow considerable savings. Every test is quantified as Euros. Management is carried out according to UNI EN ISO 9001:2008.

Conclusions: POCT network for blood gas analysis in the Health Organization of Alessandria is a proper system for decentralized tests where Central Laboratory Unit controls and monitors all activities according national and local laws. It is also a good example where a lab Technician can perform specific functions as well as management of activities, cooperation in savings resources and improving quality of patient care.

P04-11

Method validation for quantifying cobalt in serum by electrothermal atomic absorption spectrometry

López-Lozano L (1), Palazón-Bru I (1), Morales-Bayle C (1), Palazón-Bru A (2), González-Estecha M (1), Arroyo-Fernández M (1)

(1) Hospital Clínico San Carlos, Laboratory Medicine, Madrid, Spain
(2) Universidad Miguel Hernández de Elche, Departamento de Medicina Clínica, Alicante, Spain

Corresponding author: lore_l_l@hotmail.com

Background: Metal-on-metal bearings may release a variety of metal ions into local tissue and into general circulation, with Cr and Co being the most widely reported of the released metals. The aim of this study is to validate a method for cobalt determination in serum by electrothermal atomic absorption spectrometry, as it is an emerging area of interest in Laboratory Medicine.

Materials and methods: A Perkin Elmer AAnalyst 800 atomic absorption spectrometer with Zeeman correction system was used.

Instrumental conditions:

- Wavelength: 242.5 nm
- Slit width: 0.2 nm
- Measurement: peak area
- Furnace program
- Drying [temperature (°C)/time (s) ramp-hold]: 80/10-30; 130/10-30
- Pyrolysis [temperature (°C)/time (s) ramp-hold]: 800/20-20; 1200/10-20
- Atomization [temperature (°C)/time (s) ramp-hold]:1900/0-3
- Cleaning [temperature (°C)/time (s) ramp-hold]: 2400/1-4; 20/1-5
- The flow gas used in all stages, except for atomization, was 250 mL/min

Reagents:

- Standard cobalt of 1000 mcg/mL and a matrix modifier of palladium and magnesium nitrates were used.

- Sample treatment:
- Blank, standards and samples were diluted 1 to 2 with the matrix modifier.
- The calibration curve was performed with five standards: 10, 20, 30, 40 and 50 mcg/L.

Results and conclusions:

- Characteristic mass (pg): 21 pg
- Detection limit: 0.31 mcg/L
- Quantification limit: 1.04 mcg/L
- Linearity: until 50 mcg/L
- Uncertainty: ± (7.4% + 0.4 mcg/L)
- Imprecision: 2.4% + 0.2 mcg/L
- Bias: < 15%

The proposed method proves to be sensitive, robust, accurate and precise for biomonitoring the concentration of cobalt in serum samples as an indicator of health risk.

P04-12

Method validation for quantifying chromium in serum by electrothermal atomic absorption spectrometry

Palazón-Bru I (1), López-Lozano L (1), Palazón-Bru A (2), González-Estecha M (1), Arroyo-Fernández M (1)

(1) Hospital Clínico San Carlos, Laboratory Medicine, Madrid, Spain
(2) Universidad Miguel Hernández de Elche, Departamento de Medicina Clínica, Alicante, Spain

Corresponding author: irene_pb@hotmail.es

Background: Metal-on-metal bearings may release a variety of metal ions into local tissue and into general circulation, with Cr and Co being the most widely reported of the released metals. The aim of this study is to validate a method for chromium determination in serum by electrothermal atomic absorption spectrometry, as it is an emerging area of interest in Laboratory Medicine.

Materials and methods: A Perkin Elmer AAnalyst 800 atomic absorption spectrometer with Zeeman background correction system was used.

Instrumental conditions:

- Wavelength: 357.9 nm
- Slit width: 0.7 nm
- Measurement: peak area

Furnace program:

- Drying [temperature (°C)/time (s) ramp-hold]: 80/10-30; 130/10-30
- Pyrolysis [temperature (°C)/time (s) ramp-hold]: 800/20-15; 1300/20-20
- Atomization [temperature (°C)/time (s) ramp-hold]:2200/0-3
- Cleaning [temperature (°C)/time (s) ramp-hold]: 2600/1-4; 20/1-5
- The flow gas used in all stages, except for atomization, was 250 mL/min

Reagents:

- Standard chromium of 1000 mcg/ mL and a matrix modifier of palladium and magnesium nitrates were used.

Sample treatment:

- Blank, standards and samples were diluted 1 to 2 with the matrix modifier.
- The calibration curve was performed with five standards: 2,4,6,8 and 10 mcg/L.

Results and conclusions:

- Characteristic mass (pg): 7.1 pg
- Detection limit: 0.074mg/L
- Quantification limit: 0.24 mcg/L
- Linearity: until 10 mcg/L
- Uncertainty: ± (10.99% - 0.04 mcg/L)
- Imprecision: 4.59% - 0.02 mcg/L
- Bias: < 15%

The proposed method proves to be sensitive, robust, accurate and precise for biomonitoring the concentration of chromium in serum samples as an indicator of health risk.

P04-13

Palladium as a universal matrix modifier for ETAAS technique in biological samples

Palazón-Bru I, López-Lozano L, Trasobares-Iglesias E, González-Estecha M, Arroyo-Fernández M

Hospital Clínico San Carlos, Laboratory Medicine, Madrid, Spain

Corresponding author: irene_pb@hotmail.es

Background: Choosing the best matrix modifier is one of the key points in measurements by electrothermal atomic absorption spectrometry (ETAAS). It is an advantage to have a single matrix modifier for clinical laboratories that perform multi-element analyses. A mixture of palladium and magnesium nitrates was evaluated. This matrix modifier has two main mechanisms of action: it thermally stabilizes thermally and removes chlorinated interfering ions during the pyrolysis step. The aim of this study is to find a matrix modifier which can be used for measuring a variety of elements in biological samples, using aqueous standard calibrations.

Materials and methods: A Perkin Elmer AAnalyst 800 atomic absorption spectrometer was used.

Matrix modifier:

- 500 µL 10 g/L palladium nitrate
- 500 µL 10 g/L magnesium nitrate hexahydrate
- 100 µL Triton X-100
- 49 mL bidistilled water
- Standard solutions (1.000 µg/mL) of:
- Chromium
- Cobalt
- Selenium
- Lead
- Cadmium

Results and conclusions:

Chromium:

- Expected mass characteristic (pg): 7
- Obtained mass characteristic (pg): 7.1

Cobalt:

- Expected mass characteristic (pg): 17
- Obtained mass characteristic (pg): 21

Selenium:

- Expected mass characteristic (pg): 45
- Obtained mass characteristic (pg): 48

Lead:

- Expected mass characteristic (pg): 50
- Obtained mass characteristic (pg): 45

Cadmium:

- Expected mass characteristic (pg): 1.3
- Obtained mass characteristic (pg): 1.3

The results show that the Pd+Mg modifier removes the interferences of matrix and allows the use of aqueous standards and the performance of a simple sample treatment. It enables the use of only one modifier for the five elements.

P04-14

Diagnostic odds ratio as a single indicator of PCT performance in different clinical settings

Fernandez Rodriguez E (1), Barros Garcia C (1), Garcia Alonso S (1), Fernandez Garcia E (2)

(1) Cabuenes Hospital, Biochemistry, Gijon, Spain
(2) Central Hospital, Intensive Care Unit, Oviedo, Spain

Corresponding author: eloyfr@gmail.com

Background: Procalcitonin (PCT) has been most widely evaluated as biomarker to distinguish sepsis from other inflammatory conditions. However, different indicators of test performance have been used to assess the diagnostic accuracy of PCT. The aim of this study was to establish a homogeneous comparison by means of a Diagnostic Odds Ratio (DOR) as a single indicator of PCT's accuracy.

Materials and methods: Nine studies corresponding to 1 systematic review and 8 meta-analyses of sepsis in adults, sepsis in children and neonatal sepsis, were included. DOR (ratio of the of positivity in disease relative to the odds of positiv-

ity in nondiseased) was calculated as: DOR = Likelihood ratio (+)/ Likelihood ratio (-) = [Sens/(1-Spec)] / (1-Sens)/Spec] from the Sensitivities and Specificities of 59 reports. Statistical analysis was performed with the program Meta-Disc.

Results and conclusion: Tests potentially useful tend to have DOR well above 20. LH (+) greater than 10 or LH (-) less than 0.1 have the potential to alter clinical decisions. LR (+) between 5 and 10, and LH (-) between 0.1 and 0.2 often provide useful additional information. Tests with LH ranging from 0.33 to 3 rarely alter clinical decisions. The results of DOR in our study were between 6.77 (ED, adults) and 14.8 (ICU, neonates) showing that the diagnostic performance of the PCT in different settings is moderate (low to intermediate, median DOR under 20) thus not lending support to its widespread use.

P04-15

Serum copper, ceruloplasmine and zinc concentrations in a hospital working population

Castillo Perez C, González-Estecha M, Diaz Diaz A, Trasobares E, Morales C, Arroyo M

Hospital Clínico San Carlos, Laboratory Medicine, Madrid, Spain

Corresponding author: avellaneda.diaz.diaz@gmail.com

Background: Copper and zinc are essential micronutrients for humans, even though they are not included in routine analyses. The objective of this study is to measure copper and zinc in a hospital working population and to evaluate the need of measure copper and ceruloplasmine together.

Material and methods: We recruited 395 employees (64 men and 331 women). Serum copper (µg/dL) and zinc (µg/dL) concentrations were measured using flame atomic absorption spectrometry. In addition we measured ceruloplasmine (mg/dL) by immunonephelometry in the 100 non menopausal women (14 under oral contraceptive treatment).

Results: The mean of serum copper was 118.2 µg/dL (SD: 26.4). Copper percentiles (5, 25, 50, 75, 95) were: 84, 102, 114, 127, 178 µg/dL. Among the non menopausal women (100), the mean of ceruloplasmine was 36.8 mg/dL (SD: 14.4). Women under oral contraceptive treatment had serum ceruloplasmine (66.3, SD: 13.6) and copper (204.9, SD: 19.2) higher ($P < 0.01$) than those who did not take contraceptives (32.1, SD: 8.4; 108.9, SD: 20.5). However, no significant differences were found in the calculated free copper concentrations between both groups of women. The serum zinc mean was 88.3 µg/dL (SD: 10.6). Zinc percentiles (5, 25, 50, 75, 95) were: 71, 82, 88, 95, 105 µg/dL.

Conclusion: We found an appropriate copper and zinc status in this population. Since ceruloplasmine concentrations influence copper concentrations, it is necessary to measure both of them in order to correctly interpret serum copper in clinical practice. It would be desirable to establish reference values for zinc, copper and ceruloplasmine in Spanish population.

P05 – Diabetes mellitus

P05-01

Comparison of HbA1c results by two immunoturbidimetric methods

Gugo K (1), Kelava I (2), Tandara L (1), Salamunić I (1)

(1) University Hospital Center Split, Department of Medical Laboratory Diagnosis, Split, Croatia
(2) General Hospital "Hrvatski ponos", Laboratory of Medical Biochemistry and Hematology, Knin, Croatia

Corresponding author: ilza.salamunic@gmail.com

Background: The measurement of HbA1c as a biomarker for long-term monitoring of glycemic control in diabetic patients demands high analytical precision. The aim was to evaluate analytical performance of immunoturbidimetric method for measurement of HbA1c (Tina-quant Hemoglobin A1c Gen. 2) on Cobas Integra 400 plus analyzer (Roche) before introduction into routine use.

Methods: Within-run and day-to-day imprecision was determined using two commercial control samples (PreciNorm and PreciPath, Roche) and one patient sample with value near the target point for good glycemic control (7%; 54 mmol/L). Method comparison was obtained with parallel measurement of patients samples (N = 75) on Dimension Xpand (Siemens) analyzer (immunoturbidimetrical method traceable to DCCT) used routinely in our laboratory. Statistical analysis was performed using MedCalc 12.2.1. software.

Results: Coefficients of variation for within-run imprecision ranged (N = 25): PreciNorm 0.95%, PreciPath 0.82%, and patient sample 1.16%. Coefficients of variation for day-to-day imprecision ranged (N = 20): PreciNorm 0.95%, PreciPath 0.54%, patient sample 0.72%. Cumulative intralaboratory imprecision for low and high commercial controls and a patient sample was 1.4%, 1.0% and 1.3% respectively. The correlation coefficient (r) between two analyzers was r = 0.98. The parameters of Passing-Bablok regression showed systematic differences among analytical systems (95% CI, a = -1.343 to -0.400; b = 1.000 to 1,143). Systematic differences evaluated by Bland-Altman method were Cobas – Dimension (95% CI = - 0.93 to 0.28).

Conclusion: Method demonstrated high reproducibility meaning that is suitable for long-term follow-up of diabetic patients. If method is changed, constant bias should be corrected for improved results agreement.

P05-02

Renal tubular markers for predicting early stage diabetic nephropathy in patients with type I diabetes

Shabani M

University of Tyrana, Faculty of natural Science, Deparment of Chemistry, Tirana, Albania

Corresponding author: milazimshabani@hotmail.com

Background: Early detection of renal dysfunction is important since this is the first step in the progressive loss of renal functions. The aim of this study was carried out to evaluate the correlation between the duration of diabetes, metabolic control (glycemic control, HbA1c) and the urinary excretion of tubular marker: β2-microglobulin, α1 microglobulin as LMWP and lysosomal enzyme N-acetyl B-D-glucosaminidase for early detection of subclinical nephropathy.

Materials and methods: This study consisted of 46 randomly selected patients with type 1 diabetes mellitus that diagnosed in Department of Endocrinology and with a normal serum creatinine (< 80mmol/L) and no signs of clinical nephropathy. 30 healthy controls were randomly select. Biochemical parameters glucose, HbAlc, serum and urinary creatinine, creatinine clearance and urinary excretion of low molecular weight proteins and urinary N-β-D glucosaminidase were measured with standarised methods in all cases and controls.

Results: The study showed a significant increase in β-2Microglobulin ($P < 0.001$), α-1Microglobulin($P < 0.01$), NAG ($P < 0.01$), HbAlC ($P < 0.001$) in diabetic patients compared to control. There was a positive correlation between the duration of diabetes and β2-microglobulin ($P < 0.001$), NAG ($P < 0.05$), HbAlc ($P < 0.001$). There was a significant positive correlation between glycemic control (HbA1C) and β-2microglobulin, α-1microglobulin and N-acetyl-β-D-glucosaminidase ($P < 0.001$, $P < 0.01$, $P < 0.05$) respectively.

Conclusion: Tubular markers appear to be useful in early detection of diabetic nephropathy with positive correlation with the duration of type 1diabetes and glycemic control (HbA1c).

P05-03

IL-12 concentrations in the aqueous humor and serum of diabetic retinopathy patients

Sapunar A (1), Gverovic Antunica A (2), Karaman K (3), Rodin Kurtovic A (1), Bosnjak N (1)

(1) University Hospital Split, Department of Laboratory Diagnostic, Split, Croatia
(2) General Hospital Dubrovnik, Department of Oftamology, Dubrovnik, Croatia
(3) University Hospital Split, Clinic of Oftamology, Split, Croatia

Corresponding author: ada.sapunar@kbsplit.hr

Introduction: Previous studies suggest that inflammation plays an important role in pathogenesis of diabetes. Cytokines may play positive or negative role in immunological reactions. Among many cytokines, interleukin 12 (IL-12) is known to be a strong pro-inflammatory cytokine.

Materials and methods: A total of 76 participants were enrolled in this study and classified in 4 groups: 23 diabetic patients with non-treated retinopathy, 17 diabetic patients with treated retinopathy, 12 diabetic patients without retinopathy, and 24 healthy control patients. Serum and aqueous humor samples were taken for the analysis of IL-12 concentration.

Results: There was a significant difference between the groups in IL-12 concentrations in the aqueous humor ($\chi 2$ (3,N=76) = 27.137; P = 5.5 x10-6) with highest values measured in the non-treated diabetic retinopathy group (12.40 pg/mL). No significant differences in IL-12 serum concentrations between the groups were found (F = 0.405, P = 0.750). Correlation analysis of IL-12 concentrations in the serum and aqueous humor showed association between the two variables only in non-diabetic patients (P = 0.003).

Conclusion: To the best of our knowledge, this is the first study to show a significantly higher concentration of pro-inflammatory cytokine IL-12 in the aqueous humor of non-treated diabetic retinopathy patients in comparison with diabetic patients treated for retinopathy, diabetic patients without retinopathy or with healthy individuals. Because the serum levels of IL-12 did not differ considerably between the studied groups, it is possible that this is due to its local production and secretion.

P05-04

Evaluation of obesity influence on lipid profile in type II diabetes mellitus

Leitão M, Almeida A, Bellém F, Renato A, Apolónio J, Medeiros R

Escola Superior de Tecnologia da Saúde de Lisboa (ESTeSL), Clinical Analyses and Public health, Lisbon, Portugal

Corresponding author: mcleitao@estesl.ipl.pt

Background: There are an increasing number of obese people with type II diabetes mellitus (DM II) associated with dyslipidemias and cardiovascular problems. This study evaluated the obesity influence, by calculating body mass index (BMI) on lipid profile in subjects with DM II.

Materials and methods: 70 patients with DM II, aged ≥ 18 years, 35 obese (O) and 35 non obese (NO), selected from medical patient's records of Faro Hospital' Diabetology Service. Were analyzed total cholesterol (TC), LDL, HDL, triglycerides (TG), fasting plasma glucose and glycosylated hemoglobin (HbA1c). The statistical processing included the independent student t-test, linear coefficient correlation and Pearson chi-square.

Results and conclusions: The values obtained showed that mean of TC and TG are slightly higher in the group O related to NO (O: CT = 181.43 mg/dL and TG = 143.6 mg/dL; NO: CT = 180.60 mg/dL and TG = 126.3 mg/dL). LDL, HDL, fasting glucose and HbA1c, showed higher values in NO group (O: LDL = 100.9 mg/dL, HDL = 44.89 mg dL, glucose = 175.4 mg/dL, HbA1c = 7.9%; NO: LDL = 103.9 mg/dL, HDL = 46.63 mg/dL, glucose = 188.6 mg/dL, HbA1c = 8.2%). We concluded that there are significant differences between groups but obesity was not a crucial factor of influence on lipid profile, with the patients under study.

P05-05

Non-HDL cholesterol as a predictor of impaired glucose metabolism

Bergmann K, Sypniewska G

Nicolaus Copernicus University Collegium Medicum in Bydgoszcz, Department of Laboratory Medicine, Bydgoszcz, Poland

Corresponding author: bergmann@vp.pl

Background: Non-HDL cholesterol (non-HDL-C), recently used as a risk factor for cardiovascular disease and surrogate for apolipoprotein B level, may be a useful indicator of early disturbances of glucose metabolism, reflected by glycated hemoglobin (HbA1c), vitamin 25(OH)D3 and HOMA-IR values, in healthy, non-obese individuals.

Materials and methods: Study consisted of 82 healthy, non-diabetic subjects, aged 25-40 years (49 women and 33 men). HbA1c in the whole blood, plasma fasting glucose and serum total cholesterol (TC), HDL-C, triglycerides (TG), insulin, C-reactive protein (CRP), total bilirubin, apolipoproteins B100 and AI (apoB, apoA-I), vitamin 25(OH)D3 and antropometric measurements (BMI, waist to hip ratio-WHR) were performed. LDL-C, non-HDL-C, HOMA-IR and atherogenic indexes (TC/HDL-C, LDL-C/HDL-C, apoB/apoAI) were calculated. Subjects were divided by tertiles of non-HDL-C.

Results: Significantly higher values of BMI, glucose, insulin, HbA1c, HOMA-IR, total bilirubin, TG, non-HDL-C (127.6 *vs*. 141 mg/dL; $P < 0.001$) were found in men compared to women. In the whole study group lipids, except HDL-C, apoB, HOMA-IR, HbA1c, atherogenic and anthropometric indexes were increased and 25(OH)D3 was decreased in subsequent non-HDL-C tertiles. Although non-HDL-C showed the strongest correlation with traditional lipid parameters, apoB and atherogenic indexes, it has been also significantly related with WHR ($r = 0.46$; $P = 0.012$), glucose ($r = 0.42$; $P = 0.016$), HbA1c ($r = 0.38$; $P = 0.04$), HOMA-IR ($r = 0.37$; $P = 0.04$) and 25(OH)D3 ($r = -0.39$; $P = 0.027$).

Conclusion: Relationship, observed in young healthy non-obese individuals, between non-HDL-C and glucose, HOMA-IR, 25(OH)D3 and HbA1c may reflect an early impairement of glucose metabolism and the risk of diabetes and metabolic syndrome in the future.

P05-06

HbA1c – Portuguese EQAS Schemes (2003-2011)

Faria A (1), Correia H (1), Brito C (1), Gomes M (2)

(1) Instituto Nacional de Saude Dr Ricardo Jorge, Programa Nacional de Avaliação Externa da Qualidade, Lisbon, Portugal
(2) Lisbon, Portugal

Corresponding author: ana.paula.faria@insa.min-saude.pt

Objectives: Of the different objectives of the external evaluation tests, we present the performance evaluation of laboratories participating in PNAEQ, for hemoglobin A1c schemes, since 2003, with respect to the different methodologies used to determine this parameter.

Results: The methods used by participants PNAEQ since 2003 are: immunoturbidimetry, LPLC, HPLC and colorimetry, with an average participation of 21.5%, 8.1%, 60.1% and 3.2% respectively. The method of ion exchange chromatography was mentioned by the laboratories from 2004 to the present date with an average attendance of 4.2%. In 2009 the immunoinhibition method was used by only 1.7%, which did not allow statistical evaluation (number of participants less than 3). It is found that the HPLC method is used by most laboratories with increased use over the years (43% to 75%), and also has a lower CV% (pathological level of 2.7 to 5.2% and normal level of 3.9 to 10.3%), with an increase in performance in the last two years (CV% around 3.5%). For immunoturbidimetry and chromatography methods, the CV% calculated did not vary significantly with different concentrations of the samples.

Conclusion: The lower coefficient of variation was observed in the pathological level, being HPLC the

method with lowest values. For methods of chromatography and immunoturbidimetry the calculated CV% have a large variation with the concentration of the sample. It is found that the HPLC method is what is closest to the value of CV% indicated on Standard 033/2011 of Portugal 's Health General Directorate (Prescription and determination of glycated hemoglobin A1c).

P05-07

Vitamin D levels and its relationship with fasting glucose in pregnancy

Illana F (1), Castillo C (1), Perez N (2), Bausela C (1), Gaviña B (1), Torrejon M (1)

(1) Hospital Clinico San Carlos, Laboratory Medicine Department, Madrid, Spain
(2) Hospital Clinico San Carlos, Endocrinology and Nutrition Department, Madrid, Spain

Corresponding author: fran_illana@hotmail.com

Background: Vitamin D is implicated in several physiologic processes. Lower levels of Vitamin D 25-OH have been inversely associated with maternal glycemia but association with risk of gestational diabetes (GDM) are controversial. The aim of this work is to study the relationship between vitamin D levels and fasting glucose between 24-28 weeks of gestation, using a cutoff ≥ 92 mg/dL according to recommendations of the American Diabetes Association (ADA) and the International Association of Diabetes and Pregnancy Study Groups (IADGSP).

Materials and methods: 136 women were screened for gestational diabetes between 24-28 weeks during the summer months. Serum basal glucose and vitamin D were measured in an Olympus AU2700 autoanalyzer (Beckman) and Liaison (Dia Sorin), respectively. Patients were separated in groups depending on vitamin D levels (< 10 mg/dL severe insufficiency, 10-20 mg/dL insufficiency, 20-40 mg/dL deficiency and > 40 mg/dL sufficiency). Statistical analysis was performed using SPSS-15. T-test and Chi-squared test were used to compare groups.

Results: 70 pregnant women had insufficient levels of Vitamin D (< 20 ng/mL) and 24 had impaired fasting glucose values (≥ 92 mg/dL). No statistical differences were found between vitamin D levels of pregnant women with normal or impaired fasting glucose (20.6 mg/dL and 19.2 mg/dL respectively). No significant differences were found among vitamin D groups comparing diabetic and non diabetic patients.

Conclusions: In our population, no association was found between vitamin D deficiency and impaired fasting plasma glucose using cut-off 92 according to ADA criteria.

P05-08

Implications of variable preanalytical procedures for the diagnosis of diabetes mellitus in Croatia

Vučić Lovrenčić M (1), Božičević S (1), Radišić Biljak V (1), Mesić R (1), Poljičanin T (2), Metelko Ž (3)

(1) Merkur Teaching Hospital, Institute of Clinical Chemistry and Laboratory Medicine, Zagreb, Croatia
(2) Institute of Public Health, Zagreb, Croatia
(3) Merkur Teaching Hospital, Vuk Vrhovac University Clinic, Zagreb, Croatia

Corresponding author: vucic@idb.hr

Background: Preanalytical sample handling is of utmost importance for the accurate glucose results in screening and diagnosis of diabetes mellitus. The effect of in vitro glycolysis should be minimised by immediate cooling of blood samples for glucose analysis and plasma separation from the cells within 30 min from sampling. An effective glycolysis inhibitor is recommended for any delayed processing. The aim of this study was to evaluate current practice regarding preanalytical steps of glucose measurement in Croatian laboratories.

Materials and methods: A structured, anonymous survey has been conducted among the participants of the Croatian Chambers of Medical Biochemists' continuous education course „The role of laboratory in diagnosis and management of diabetes melli-

tus" in September 2011. The survey considered: type of primary sample, use of glycolysis inhibitors, sampling location, sample storage conditions and average time of plasma separation after venipuncture.

Results: A total of 56 participants (32%, 30%, 11% and 11% from the primary, county- and teaching-hospitals, and private laboratories, respectively) were included in the survey. Venous serum was the most prevalent primary sample type (62%), and glycolysis inhibitors were used in 22% of the laboratories. Laboratory only, and combined laboratory and family physician's office were equally reported (43%) as the sampling location. Plasma/serum separation within 30 min is performed in 34% of the laboratories, whereas delayed processing (30-60 min, 1-2 h and >2 h) was reported by 37%, 20% and 9% of the laboratories, respectively.

Conclusions: Preanalytical procedure for glucose measurement in Croatia urgently needs improvement and harmonization.

P05-09

Use of urinary protein:creatinine ratio in advanced stages of chronic kidney disease in diabetes

Radišić Biljak V (1), Božičević S (1), Pavković P (2), Vučić Lovrenčić M (1)

(1) Merkur Teaching Hospital, Institute of Clinical Chemistry and Laboratory Medicine, Zagreb, Croatia
(2) Merkur Teaching Hospital, Department of Nephrology, Zagreb, Croatia

Corresponding author: Vanja.Radisic.Biljak@idb.hr

Background: Albumin:creatinine ratio (ACR) is the recommended method for the detection of incipient kidney disease in diabetic patients. However, protein daily excretion rate is used for the quantification and monitoring of proteinuria in more-advanced stages of chronic kidney disese. We wanted to determine whether measurement of protein:creatinine ratio (PCR) can be used as an alternative to protein excretion rate in detecting clinically significant proteinuria in diabetic patients.

Materials and methods: 48 diabetic patients were included in the study. We measured ACR and PCR in random urine samples and protein contents in 24-h urine samples. All measurements were made on Olympus AU600. Urinary albumin was measured by immunoturbidimetric method, while urinary creatinine and protein were measured by the spectrophotometric Jaffe and pyrogallol red method, respectively. Clinically significant proteinuria was considered to be present with the ACR ≥ 30 mg/mmol which is approximately equivalent to PCR 50 mg/mmol. All results were analyzed with MedCalc 9.4.2.0. statistical software (MedCalc Software bvba, Mariakerke, Belgium).

Results: ACR was ≥ 30 mg/mmol in 28/48 diabetic patients. Sensitivity was 100% and specificity was 95.0% for PCR as an indicator of clinically significant proteinuria at a cut-off value of 50 mg/mmol. Protein daily excretion rate as an indicator of clinically significant proteinuria gave lower values for sensitivity and specificity at the cut-off value of > 0.5g/24h; they were 78,6% and 85,0% respectively.

Conclusions: The protein:creatinine ratio performed better as an indicator of clinically significant proteinuria than the 24-urine collection method in diabetic patients.

P05-10

Relationship between PAI and CRP in diabetic patients

Milić M, Marošević L, Paradinović K, Šerić V

KBC Osijek, Department of Clinical Laboratory Diagnostics, Osijek, Croatia

Corresponding author: milic.marija@kbo.hr

Background: CRP is a risk marker of cardiovascular disease and his role in promoting atherosclerosis has been recently investigated. Previous studies show that CRP and hyperglycemia induce PAI-1 expression and suggest PAI-1 to cause a hypofibrinolitic state in atherosclerotic plaques. Therefore, we investigated CRP, PAI and other inflammatory parameters in diabetics.

Materials and methods: Forty-one diabetic (aged 65 ± 8.95) patients with HbA1c (average 9.98%; range 5.9-15.6) were enrolled. We analyzed routine inflammatory parameters (CRP, WBC, ESR, fibrinogen) and compared them with PAI activity. We excluded diabetics with CRP > 20 µg/L. Statistical analysis was performed by SPSS 19 (IBM, Armonk, New York, SAD).

Results: PAI showed significant relationship with CRP in diabetic subjects (r = 0.303; P = 0.05). No correlation was found between PAI and other inflammatory parameters. PAI did not correlate with HbA1c. Previous research showed that hyperglycemia increased PAI expression in cell cultures but that has not been confirmed in our study.

Conclusion: Previous studies have demonstrated that CRP induces PAI expression in human and bovine aortic endothelial cells. Our results show correlation between CRP and PAI in our patients. If further research confirm these findings, it is possible that PAI will be recognized as a novel risk marker of cardiovascular disease in diabetes mellitus.

P05-11

Tissue transglutaminase antibodies and HLA haplotypes in type 1 diabetic Lithuanian children

Skrodeniene E (1), Vitkauskiene A (1), Sadauskaite-Kuehne V (2), Bilbao J (3), Padaiga Z (4), Ludvigsson J (5)

(1) Lithuanian University of Health Sciences, Department of Laboratory Medicine, Kaunas, Lithuania
(2) Parkwayhealth Medical Center, Pediatric, Shanghai, China
(3) University of Basque Country , Endocrinology and diabetes Research Group, Baracaldo, Spain
(4) Lithuanian University of Health Sciences, Department of Preventive Medicine, Kaunas, Lithuania
(5) Linkoping University, Department of Clinical and Experimental Medicine, Linkoping, Sweden

Corresponding author: erika.s@takas.lt

Introduction: Type 1 diabetes (T1D) is an autoimmune disease. Celiac disease (CD) is the second most prevalent autoimmune condition accompanying T1D. It may be partially explained by shared genetic susceptibility to HLA haplotypes. The aim of our work was to determine prevalence of tissue transglutaminase antibodies (TTG Ab) in T1D and healthy control children and to assess whether positivity of TTG Ab is related to CD associated HLA haplotypes.

Materials and methods: 271 children with T1D and 289 healthy control children were serologically screened for the presence of TTG Ab IgG class. Genetic testing was performed for 30 children with T1D and positive TTG Ab, 137 children with T1D and negative TTG Ab, and 4 TTG Ab positive control children.

Results: 11.1% of the diabetic children and 1.4% healthy controls were found to be positive for TTG Ab (P < 0.001). HLA DR3-DQA1*0501-DQB1*0201 or/and DR4-DQA1*0301-DQB1*0302 were found in 93.3% of TTG Ab positive and in 77.4% TTG Ab negative diabetic children (P = 0.047). There were no significant differences in the frequency of heterozygotic DR3-DQA1*0501-DQB1*0201 and DR4-DQA1*0301-DQB1*0302 haplotype between TTG Ab positive and TTG Ab negative diabetic children. DR4-DQA1*0301-DQB1*0302 homozygotic haplotype was found in 6.7% of TTG Ab positive but wasn't found in TTG Ab negative diabetic children (P = 0.002). All (N = 4) TTG Ab positive control children were heterozygotic for DR3-DQA1*0501-DQB1*0201 haplotype (P < 0.05).

Conclusions: This study showed higher prevalence of TTG Ab in children with T1D than in their healthy counterparts. TTG Ab positive diabetic children significantly more frequently had homozygotic DR4-DQA1*0301-DQB1*0302 haplotype and control children – heterozygotic DR3-DQA1*0501-DQB1*0201 haplotype.

P05-12

Bariatric surgery treatment for type 2 diabetes

Mirošević G (1), Kruljac I (1), Nikolić M (2), Butorac D (3), Ljubičić N (2), Vrkljan M (1)

(1) University Hospital Centre "Sestre milosrdnice", Division of Endocrinology, Diabetes and Metabolic Diseases, Department of Internal Medicine, Zagreb, Croatia
(2) University Hospital Centre "Sestre milosrdnice", Division of Gastroenterology and Hepatology, Department of Internal Medicine, Zagreb, Croatia
(3) University Hospital Centre "Sestre milosrdnice", Department of Gynecology and Obstetrics, Zagreb, Croatia

Corresponding author: goranamirosevic1@net.hr

Background: Bariatric surgery treatment should be considered in cases of extreme obese patients with type 2 diabetes, who could not reduce body mass through physical activity, diet and pharmacotherapy.

Materials and methods: There are two female patients shown with type 2 diabetes regulated by oral hypoglycemic agents, who were subjected to gastric sleeve resection.

Results: A 55 year old patient had BMI of 43.5 kg/m^2, blood glucose 10 mmol/L, HbA1c 7.4 %, score for beta-cell function assessment (HOMA-B) 34%, preoperative insulin sensitivity (HOMA-S) 56.4%, ghrelin level 807.7 pg/mL, and leptin level 35 ng/mL. A year after surgery BMI was reduced to 32.7 kg/m^2, whereas blood glucose (5.9 mol/l) and HbA1c (6%) were normalized without hypoglycemic agents. HOMA-B increased to 95% and HOMA-S to 60.1%. Ghrelin increased to 864 pg/mL and leptin decreased to 8.1 ng/mL.

A 44 year old patient had BMI 44.9 kg/m^2, blood glucose 8.2 mol/L, HbA1c 6%, HOMA-B 51%, HOMA-S 55.5%, ghrelin 603 pg/mL, and leptin 29.3 ng/mL. A year after surgery BMI was reduced to 35.3 kg/m^2, whereas blood glucose (4.3) and HbA1c (4%) were normalized without hypoglycemic agents. HOMA-B increased to 130.1% and HOMA-S to 102.6%. Ghrelin increased to 1008 pg/mL and leptin decreased to 12.3 ng/mL.

Conclusions: Reduction of body mass leads to improvement of pancreatic beta cell function and therefore better diabetes control. Bariatric surgery is a promising method for treating diabetes in obese patients.

P06 – Education

P06-01

Do we need complex education courses in special fields of laboratory medicine?

Vavilova T, Sirotkina O, Gaikovaya L

North-West State Medical University named after Mechnikov, Laboratory Department, Saint-Petersburg, Russia (Russian Fed.)

Corresponding author: vtv.lab@rambler.ru

Objective: The fast progress in knowledge about coagulation disorders and new pharmacological agents for treatment and haemorrhagic and thrombotic events are the reason for complex education for clinicians and laboratory staff.

Materials and methods: From Sept 2006 up to May 2012 we organized 10 CME courses “Clinical and laboratory aspects of coagulation disorders” 72 h duration (Lectures – 26 h; Seminars + exam – 24 h; Practical classes in laboratory and clinical departments – 22 h).

Results: 58 persons were educated – 38 laboratory professionals and 20 clinicians (cardiologists, gynaecologists, internists, clinical pharmacologist) from 8 cities and 34 medical centres. The better experience was to educate both – laboratory staff and clinician from the same medical centre in the same time. One year after finishing the course the questionnaire was sent to participants: the usefulness were marked by 94%, “important for every day work” – 86%, “wish to repeat in a 5 years” – 93% of responders. The more important themes: genetics of thrombophilia, laboratory testing in acquired and inherited bleeding and thrombotic disorders (risk factors, reasons, predisposition), DIC, laboratory control of antithrombotic and anti-

platelet therapy, new anticoagulants, discussions around the clinical cases, patient's examination.

Conclusion: Complex education in special fields of laboratory medicine for clinicians and laboratory staff is the important part of postgraduate CME. It allows improving clinical work in medical centres, especially in difficult and unusual clinical situations.

P06-02

Preparing medically oriented education of scientific staff in line with the EC4RC syllabus

Wallinder H (1), Pettersson T (2), Ohlsson M (3), Bökman F (4), Olsson L (5), Gälman C (6)

(1) Swedish Association of Clinical Biochemists, Aleris Medilab, Clinical Chemistry, Stockholm, Sweden
(2) Swedish Association of Clinical Biochemists, LeanLabMed, Medical Diagnostics, Stockholm, Sweden
(3) Swedish Association of Clinical Biochemists, Sahlgrenska University Hospital, Clinical Chemistry, Gothenburg, Sweden
(4) Swedish Association of Clinical Biochemists, Falu Lasarett, Clinical Chemistry, Falun, Sweden
(5) Swedish Association of Clinical Biochemists, Örebro University Hospital, Clinical Chemistry, Örebro, Sweden
(6) Swedish Association of Clinical Biochemists, Karolinska University Laboratory, Clinical Chemistry, Stockholm, Sweden

Corresponding author: Hans.Wallinder@aleris.se

Background: Healthcare and medical diagnostics are in transformation enforced by growing patient demands but shrinking financial recourses simultaneously in a process of translating expanding biological knowledge and emerging new technologies into valuable medical practices. Educational programs in medical diagnostics must adopt new diagnostic focus, scientific and technical knowledge, service oriented operational skills and business oriented management. Multidisciplinary trends and system thinking in healthcare services add collaborative dimensions into the practice of medical diagnostics. A gap in Swedish laboratory medicine is lack of organized medically oriented training of the scientific staff. The Swedish Association of Clinical Biochemists has initiated an effort to prepare a multidisciplinary educational program to improve the scientific knowledge base in support of diagnostic service excellence.

Methods:

1) Systematic investigation of the present status among scientific staff to fully understand variation
2) Benchmark the present status knowledge base with that suggested in the EC4RC syllabus
3) Investigate future healthcare demand to envision scientific and corresponding diagnostic qualifications
4) Establish collaboration with the medical and operational staff in design of educational program
5) Establish road map for change and agreement on methodology for progress.

Results: The present status is evaluated against the EC4RC syllabus. A holistic approach to future medical diagnostic services is formulated and communicated as well as a strategic approach regarding the role of the scientific staff.

Conclusion: The role of the scientific staff in Swedish laboratory medicine will be defined through dedicated training program and integrated into the Swedish Model of Medical Diagnostic Practice.

P06-03

Education in laboratory medicine at Lithuanian university of health sciences

Urboniene D, Skrodeniene E, Vitkauskiene A

Lithuanian University of Health Sciences, Department of Laboratory Medicine, Kaunas, Lithuania

Corresponding author: daivaurboniene@yahoo.co.uk

Lithuanian University of Health Sciences (LUHS) is a State University of the Republic of Lithuania, established in Kaunas in 1922. It is the largest institution providing university degrees, training and research in biomedical sciences in Lithuania. Laboratory medicine at LUHS covers education of Laboratory Medicine Physicians and other healthcare professionals, including undergraduate, postgraduate

and continuing education, PhD studies. Professionals of Department of Laboratory Medicine take considerable part in field of laboratory medicine within different study programmes. Qualification of Laboratory Medicine Physician is obtained by specialist after multidisciplinary Laboratory Medicine residency study programme (4-years duration) following integrated Medicine study programme (6-years duration; degree – Master in Medicine; qualification – Medical Doctor). Laboratory Medicine residency study programme includes different fields of laboratory medicine: general questions of laboratory medicine, clinical chemistry, laboratory hematology, general tests and cytology of body fluids, laboratory immunology and genetics, clinical microbiology and ect. Study programme is designed to meet the European Union (EU) and United States Medical Licensing Examination (USMLE) standards with their internationally accredited exams. European Credit Transfer System (ECTS) is applied. The LUHS's degrees are recognized in the countries of the EU, USA, Lebanon, Israel, India, Turkey, Jordan, etc.

P06-04

Transnational Project: Developing Good Practice in Preregistration Training (UK & Malta)

Martin J (1), Wilson G (2), Adkins A (3), Cobley A (4), Hudson J (5), Khan A (6)

(1) University of Wolverhampton, Biomedical Science and Physiology, Wolverhampton, United Kingdom
(2) Cheltenham General Hospital, Biochemistry, Cheltenham, United Kingdom
(3) University of Birmingham, Clinical Immunology Service, Birmingham, United Kingdom
(4) City Hospital, Haematology, Birmingham, United Kingdom
(5) New Cross Hospital, Microbiology, Wolverhampton, United Kingdom
(6) Nuffield Hospital, Microbiology, Leamington Spa, United Kingdom

Corresponding author: j.martin@wlv.ac.uk

Background: The European Commission Leonardo da Vinci Mobility funding stream awarded a grant to enable 17 training officers from the UK to visit the Mater Dei Hospital and the University of Malta (UoM).

Materials and methods: Over two years, four teams of training officers each spend one week in Malta undertaking comparative analysis of the delivery of training to preregistration biomedical scientists so that new methods of good practice and innovative delivery for training can be developed.

Results: Aspects of good practice included, involvement of different grades of hospital laboratory staff in delivery of practical sessions at UoM; dedicated pathology teaching laboratories equipped with state of the art equipment; training officers who are also part-time lecturers within the UoM delivering basic laboratory skills training to the whole student group prior to them spending time within the hospital laboratory, provision of mock training areas where students can use simulated patient samples and training microscopes connected to large display screens. Innovative methods for training included streamlining generic aspects of competence assessments, establishing a transnational biomedical science training network/pathology e-learning site including various laboratory tests, virtual lectures, podcasts and shared resources for CPD activities, provision of mock training areas particularly for specimen reception, integration of theory and practice and ensuring that standard working laboratory practices are developed at an early stage in University practical classes.

Conclusion: Experiencing training within a different European setting has enabled training officers to share good practice, suggest innovative delivery of training and to fulfil their own personal objectives.

P07 – Endocrinology

P07-01

Indirect estimation for reference intervals of thyroid parameters

Milinkovic N, Ignjatovic S, Majkic-Singh N

Clinical Center of Serbia, Center of Medical Biochemistry, Belgrade, Serbia

Corresponding author: nedanvkvc@yahoo.com

Background: Reference intervals are essential for clinical laboratory test interpretation and patient care. Methods for estimating them are expensive, difficult to perform and often inaccurate. To establish indirect reference intervals from intra-laboratory data bases is an adequate alternative.

Materials and methods: All results for thyrotropin (TSH), total and free thyroxine (T4 and fT4), total and free triiodothyronine (T3 and fT3) that were stored in our laboratory information system between 2008 and 2011 were included in this study. After a logarithmic transformation of the raw data, outliers were excluded. Non-parametric reference intervals were estimated statistically after visual observation of the distribution using stem-and-leaf plots and histograms. A standard normal deviation test was performed to test the significance of difference between sub-groups.

Results: We calculated combined reference intervals for all thyroid parameters because there was no significant difference in serum values between male and female. However, we found significant difference for TSH values between ambulatory and hospitalized patients only in 2011. Indirect reference values for TSH, T4, fT4, T3 and fT3 were 0.42-3.67 mIU/L, 66.0-136.10 nmol/L, 10.20-18.40 pmol/L, 1.10-2.39 nmol/L, 3.17-5.59 pmol/L, respectively.

Conclusions: Indirect determination for laboratory-specific reference intervals using patient laboratory data values is relatively easy and cheap method. Also indirect reference intervals are more precise and true reference values of thyroid parameters for the analyzed population.

P07-02

Does TSH within normal range affect bone turnover in postmenopausal women with osteoporotic fracture

Sypniewska G (1), Pater A (1), Nowacki W (2)

(1) Collegium Medicum Nicolaus Copernicus University, Dept. of Laboratory Medicine, Bydgoszcz, Poland
(2) Collegium Medicum Nicolaus Copernicus University, Department of Orthopaedics and Traumatology, Bydgoszcz, Poland

Corresponding author: grazynaodes@interia.pl

Background: Recent studies indicated the existence of the relationship between TSH and fractures independently of thyroid hormone levels, age and bone mineral density. We aimed to evaluate the association between serum TSH and biochemical bone turnover markers in postmenopausal women with normal thyroid function, which suffered osteoporotic fracture, to answer whether the differences in TSH concentration within the reference range may affect bone metabolism.

Materials and methods: 36 women (55-93 years old) admitted to the Hospital after osteoporotic hip fracture participated in the study. Serum propeptide of type I procollagen (PINP; for postmenopausal women < 45.0 ng/mL), a bone formation marker and crosslinked C-terminal telopeptides (CTX-I; cut-off 0.439 ng/mL), a bone resorption marker and TSH were assayed. Study group was divided in two groups according to age: 55-70 and 71-93 yrs.

Results: In the older group a tendency to higher TSH concentration (Me 0.98 *vs*. 0.56 mIU/mL; $P < 0.06$) and higher median PINP (35.6 *vs*. 26.7 ng/mL; $P < 0.004$) was observed. PINP correlated negatively with TSH ($r = -0.44$ $P < 0.04$) only in the older group. Interestingly, most of women with fractures ($N = 25$; 69.4%), independently of age, had TSH concentration in the first tertile (0.36-1.38 mIU/mL) whereas only 2 (5.6%) had TSH in the highest tertile (2.4-3.41 mIU/mL). In both age groups median PINP, but not CTX-I, was the highest in patients with TSH in the first tertile.

Conclusion: In postmenopausal women TSH concentration within the reference range seems to be associated with changes in bone turnover and frequency of fractures independent of age.

P07-03

Serum calcium: much more than "bones"

Cachapuz I (1), Branco A (2), Silva S (2), Magalhaes C (1), Costa M (2)

(1) Matosinhos Local Health Unit , Clinical Pathology Service, Oporto, Portugal
(2) Matosinhos Local Health Unit, Nephrology Service, Oporto, Portugal

Corresponding author: cachapuz.isabel@gmail.com

Introduction: The giant parathyroid adenoma manifests in different forms: from asymptomatic to hypercalcaemic crisis, a rare endocrine disruption.

Case: The authors present the case of a 62 year-old man in bad general condition, asthenia, polyuria, and polydipsia with 2 months of evolution. Later the patient revealed intense pain in the lumbar spine, and presented at the emergency service. He was conscious, oriented and revealed slow speech, memory disorders and hypertension (186/98 mmHg). In analytical terms he showed: severe hypercalcemia (total serum calcium = 20.0 mg/dL), the highest value ever registered in our laboratory; ionized calcium of 2.81 mmol/L; normal albumin levels, renal insufficiency (creatinine = 3.9 mg/dL, urea = 105 mg/dL); hyperphosphataemia 5.2 mg/dL. Calcium levels decreased significantly with strong hydration and the administration of bisphosphonates. There was an adequate diuretic response, with gradual improvement of the renal function. The cervical ultrasound scan described multinodular goiter, voluminous nodule of 32 x 27 mm at the left inferior pole and PTH = 1000 pg/mL. The patient underwent an urgent cervical surgery with exeresis of the giant parathyroid adenoma (21 grm; 3cm its largest axis) and treatment to restore normal levels of serum calcium by post-surgery "hungry bone". The patient progressed, showing clinical and analytical improvement.

In this case, even though the huge serum calcium values suggested the possibility of neoplasic/metastatic disease, the final diagnosis was primary hyperparathyroidism due to the giant parathyroid adenoma that was promptly treated.

P07-04

Tear cytokines in Basedow-Graves ophthalmopathy

Koszegi T (1), Meiszterics Z (1), Mezosi E (2), Ludany A (1), Kovacs G (1)

(1) University of Pecs, Institute of Laboratory Medicine, Pecs, Hungary
(2) University of Pecs, Internal Medicine, Pecs, Hungary

Corresponding author: koszegit@freemail.hu

Background: Human tear is rich in cytokines. Even in healthy individuals the cytokine:total protein ratio is higher than that in serum. However, in Basedow-Graves disease an elevation of serum cytokine levels was found and also an altered cytokine response of the intra- and periorbital tissues was postulated. In our present study we focused on the measurement of IL-6, IL-8 and TNF-α levels and the amount of a lipocalin-type protein (orosomucoid) in tear samples of Graves patients.

Matrials and methods: Tears were collected by the Schirmer method from 35 patients and 20 control individuals without autoimmune and thyroid diseases. 80% of them were females. The mean age was 45 (25-71) years. The total tear protein content was measured by the Bradford method and tear proteins were separated by SDS-PAG electrophoresis. Tear orosomucoid was detected by chemiluminescent Western blot technique. Tear cytokine levels were quantified by an automated chemiluminescence immunoassay method (Immulite, Beckman-Coulter).

Results: TNF-α and IL-6 increased significantly (mean ± SEM, $P < 0.01$) in tear samples of the patients (TNF-α: 513 ± 57 *vs*. 217 ± 27pg/mg, IL-6: 39 ± 6 *vs*. 12 ± 1.2 pg/mg protein, patients *vs*. control in-

dividuals, respectively). In contrast, IL-8 and orosomucoid levels were similar in the two groups.

Conclusions: In our opinion increased tear TNF-α and IL-6 levels are considered to be a new finding in Graves patients. Further studies are needed to decide if tear cytokines might be used in assessment of activity of this disease and/or to monitor the efficiency of treatment. The work was supported by SROP-4.2.1.B-10/2/KONV-2010-0002 grant.

P07-05

Determination of total prolactin and prolactin treated with 25% PEG

Županić D, Crnokrak S, Mujagić R, Honović L

Pula General Hospital, Department of Laboratory Diagnostics, Pula, Croatia

Corresponding author: danijela.zupanic@pu.t-com.hr

Introduction: Prolactin is a hormone of the anterior pituitary gland and occurs in human serum in several molecular forms: monomer (predominant form), big-PRL (dimer) and big-big-PRL or macroprolactin (compound of PRL and IgG), which is biologically *in vivo* inactive and biologically active *in vitro*, and leads to apparent hyperprolactinemia leading to inadequate diagnosis, treatment and diagnostic procedures.

Objective: Our objective was to compare the values of prolactin by two different immunochemical methods and examine their sensitivity to macroprolactin by determination of prolactin in the supernatant after treating the sample with 25% PEG solution.

Materials and methods: The study included 33 patients (31 women and 2 men) with elevated prolactin values. Values of total prolactin and prolactin after precipitation with 25% PEG were determined by immunoassay on analyzers COBAS e601 (Roche, USA) and i2000SR ARCHITECT (Abbott, USA).

Results: High values of prolactin were obtained and confirmed by both immunochemical analyzers but the COBAS e601 indicates greater sensitivity to macroprolactin. If the criterion of acceptance for macroprolactin is PRLtreated / PRLtotal < 40% then macroprolactin in the study group was found in 3 patients (27%, 19% and 38%) which has been confirmed by both methods.

Conclusion: Based on the results of this study we conclude that the measured values of prolactin are above the upper limit of the reference interval on both analyzers and that COBAS e601 exhibits greater sensitivity to macroprolactin, which is consistent with literature data.

P07-06

Reference intervals for reproductive hormones in prepubertal children on the Cobas e 411 analyzer

Zec I, Tišlarić-Medenjak D, Bukovec Megla Ž

University Hospital Center "Sestre milosrdnice", Laboratory of Endocrinology, Clinics of Oncology and Nuclear Medicine, Zagreb, Croatia

Corresponding author: ivana.zec@kbcsm.hr

Background: Serum values of luteinizing hormone (LH), follicle-stimulating hormone (FSH), gonadal hormones, as well as sex-hormone binding globulin (SHBG) levels in children of prepubertal ages are essential biochemical markers used for the evaluation of suspicious disorders of sexual development. The aims of this study were to establish reference intervals for LH, FSH, E2, P, T (total and free) and SHBG in prepubertal children according to age and gender and to assess age- and gender-related differences. We used the most recent guidelines of Clinical and Laboratory Standards Institute (CLSI) to establish and verify the reference intervals.

Materials and methods: A total of 966 children, 495 girls and 471 boys, between 1 to 11 years have been participated in the study. The hormone concentrations were measured by electrochemiluminscence immunoassay on the Automated Roche cobas e 411 Analyzer. The 2.5th and 97.5th percentiles are used to form the 95% reference limits.

Results: Median values of LH, FSH and T were significantly higher in subgroups ranging from ≥ 8 to < 11 years, for both genders. In girls of that age, reference values of E2 were significantly higher than in younger ones, and in boys of the corresponding age.

Conclusions: We provided reliable reference intervals for hormones relevant for the assessment of pituitary-gonadal axes in healthy children of prepubertal age. The reference intervals have been derived out of population large enough to create age subgroups which are appropriate to express subtle changes of hormones from early childhood to the period preceding the onset of puberty.

P07-07

Seasonal variation of neonatal thyrotropin values

Gligorovic Barhanovic N (1), Samardzic M (2), Cutovic N (2), Antunovic T (1)

(1) Clinical Center of Montenegro, Center for Clinical-Laboratory Diagnostic, Podgorica, Montenegro
(2) Clinical Center of Montenegro, Institute for children disease, Podgorica, Montenegro

Corresponding author: najdana.gligorovic@kccg.me

Background: Seasonal variation in incidence of higher thyroid stimulating hormone (TSH) values in newborns has been suggested in number of geographical areas and not observed in others. Aim of this study was to evaluate seasonality of TSH levels in our National newborn screening program for congenital hypothyroidism.

Materials and methods: TSH levels were measured in all neonates between third and sixth days after birth using a blood spot assay (DELFIA Neonatal TSH assay, PerkinElmer/Wallac Oy). The data from three consecutive years were available, 2008-2010, when 24515 newborns were screened. Children with TSH values ≥ 10 mU/L were recalled for measurement of serum TSH and fT4.

Results: Recall rate for chosen time period was 1.13%, 0.97% for 2008, 1.36% for 2009 and 1.06% for 2010. Positive predictive values were 2.53% for 2008, 7.08% for 2009, 3.49% for 2010 and 4.68% for all three years. Frequency of newborns with TSH levels ≥ 10 mU/L was higher in all childbirth centers in winter comparing to summer periods. The incidence was significantly higher in january and february (1.02%) compared to july and august (0.62%), P < 0.05. Recall rates were lower in summers (0.92%, 0.34% and 0.55%) compared to winters (1.27%, 0.94% and 1.35%) for all three years.

Conclusion: Influence of environment factors on thyroid physiology was proven from many studies. There is no data regarding urinary iodine excretion over different seasons for our population but it is possible that different iodine intake during periods can contribute to seasonality of neonatal TSH values.

P07-08

Relationship between body mass index, mercury and selenium levels in a hospital working population

Morales C, González-Estecha M, Diaz Diaz A, Trasobares E, Castillo Perez C, Arroyo M

Hospital Clínico San Carlos, Laboratory Medicine, Madrid, Spain

Corresponding author: avellaneda.diaz.diaz@gmail.com

Background: Body mass index (BMI) is a simple weight-for-height index that is commonly used to classify underweight, overweight and obesity in adults. The objective of this study is to explore the relationship between blood lead and mercury and serum selenium concentrations and the BMI.

Materials and methods: We recruited 395 employees (64 men and 331 women). Blood mercury concentration (µg/L) was measured by atomic absorption spectrometry and thermal decomposition amalgamation. Serum selenium concentration (µg/L) was measured by electrothermal atomic absorption spectrometry. BMI is defined as the weight in kilograms divided by the square of the height in metres (kg/m^2).

Results: The median of blood mercury was 8.00 µg/L (IQR: 5.20-11.60). The mean of serum seleni-

um was 79.5 µg/L (SD: 11.7). The mean BMI of the population was 24.4 kg/m^2 (SD: 3.9). No significant differences were found between BMI and mercury ($r = 0.083$; $P = 0.116$) or between selenium and the selenium/mercury ratio. Considering BMI subgroups (< 25, 25-30 ≥ 30), we found a statistically significant ($P = 0.014$) increase in serum selenium concentrations (78.5 SD: 12.1; 79.8 SD: 10.7; 85.0 SD: 11.4 respectively). However, the increase observed in blood mercury concentration and selenium/mercury ratio was not significant.

Conclusions: In spite of the relation between mercury and selenium concentrations, with respect to BMI, we only found an association with serum selenium. Further studies are needed to understand the role that these trace elements, present in certain nutrients such as fish, may play in overweight and obesity.

P07-09

Is there any justification in the increasing demand for thyroid tests in our laboratory?

Fernandez Leivas A, Garcia Moreira V, Peredo Lopez B, Martinez Gago M, Garcia Alonso S, Fernandez Rodriguez E

Hospital de Cabueñes, Servicio de Analisis Clinicos, Gijon, Spain

Corresponding author: anafleivas@hotmail.com

Introduction: Thyroid disorders are among the most prevalent problems in clinical endocrinology. A majority of these patients are managed in primary care. The aim of our study was to evaluate the diagnostic performance of the increased thyroid test requests in the Health Area V of Asturias (Spain) during the last 9 years.

Materials and methods: Data were retrospectively collected by reviewing analysis of TSH, FT4 and FT3 during the last 9 years (2003-2011), from our Laboratory Information System Lmx (Siemens). These were divided into 2 groups: Group1: requests by Endocrinology Department and Group2: hospitalization, Emergency-Room and Primary Care.

Results: Progressive increase in thyroid test requests, from 44.932 TSH (2003) to 78.560 (2011), represents 84% of overall increase, which is not justified by the population increase experienced in our Area (2.75%). While the number of requests from the group 1 remained stable over the years (4.928 in 2003 and 4.811 in 2011), requests from the group 2 increased significantly (40.004 to73.749). Percentage of pathological TSH remains relatively stable at around 15% (13.8% to15.3%). Except in subclinical hypothyroidism, where its prevalence has grown from 4.94% to 10.8%, prevalences of other thyroid disorders remained constant: clinical hypothyroidism (1.28% to 0.82%), clinical hyperthyroidism (0.91 to 0.42%) and subclinical hyperthyroidism (3.89% to 2.71%).

Conclusions: We found that the increase of 84% of TSH is not then reflected in increased pathology in the endocrinology department because the pathology that increases is essentially subclinical hypothyroidism (Δ5.85%), whose patients are followed by primary care. Prevalence of thyroid diseases reported here is substantial and confirms previous reports in other populations.

P07-10

Diagnostic value of anti-thyroglobulin antibodies in the detection of thyroid pathology in children

Fernandez Leivas A, Garcia Moreira V, Blanco Cristobal C, Martinez Gago M, Garcia Alonso S, Fernandez Rodriguez E

Hospital de Cabueñes, Servicio de Analisis Clinicos, Gijon, Spain

Corresponding author: anafleivas@hotmail.com

Background: The determination of anti-thyroid peroxidase antibodies(anti-TPO) is the most specific and sensitive test for detecting autoimmune thyroid disease. Anti-TPO are commonly associated with Hashimoto's thyroiditis (90%) and Graves disease (70%), an antibody titer can be used to assess disease activity in patients that have developed such antibodies. Thyroglobulin antibodies (anti-TG) are mainly used for monitoring thyroid cancer. The determination of anti-TG in adults is

only done in the suspicion of interference in thyroglobulin measurement and thyroid scintigraphy studies. The aim of our study was to establish the diagnostic value of the isolated use of anti-TG in the pediatric population of our country.

Materials and methods: We retrospectively studied 348 samples with anti-TPO and anti-TG analysis, made during the last 6 years in children with suspected thyroid dysfunction or thyroid autoimmune disease mother (mean 12 years, range: 1 day-13 years). Data were extracted from our computer laboratory manager Lmx (Siemens). Tests were performed by fluorinmunoanalisis (Phadia Unicap).

Results: Of all requests reviewed, 34 (9.77%) had positive anti-TPO (>60 U/mL): of these, 18 were anti-TG positive (> 280 U/mL) and 16 negative. In addition, 11 (3.16%) were anti-TPO negative and anti-TG positive. In reviewing the medical history of patients with anti-TPO negative and high anti-TG, we observed that 100% had thyroid disease: 18.18% autoimmune thyroid disease inherited by the mother, 63.64% euthyroid autoimmune thyroiditis and 18.18% subclinical hypothyroidism.

Conclusions: According to the results, the quantification of anti-TG rises by 32.3% the diagnostic yield of childhood thyroid diseases (34 positive anti-TPO positive+11 only positive anti-TG) which would justify the joint determination of both antibodies in the population study.

P07-11

Testosterone quantification in human serum using liquid chromatography with tandem mass spectrometry

Depoorter M, Djuidjé C, Delongie K, Wallemacq P, Gruson D, Haufroid V

Saint-Luc Hospital, Catholic university of Louvain, Department of clinical chemistry, Brussel, Belgium

Corresponding author: depoorter.maxime@hotmail.com

Background: Evaluation of testosterone levels is important for the diagnostic and follow-up of male hypogonadism as well as female hyperandrogenism. Liquid chromatography with tandem mass spectrometry (LC-MS/MS) is one of the most specific and sensitive technique available in clinical laboratories. The objective of our work was to develop and validate a LC-MS/MS method for testosterone measurement in serum and process to its comparison with immunoassay method.

Materials and methods: The analysis was performed on an Agilent 6460 Triple Quad LC-MS/MS spectrometer with electrospray ionization (ESI). A liquid/liquid extraction (LLE) was used for sample preparation. The calibration curve ranged from 0.025 ng/mL to 50 ng/mL. The method comparison was performed with 80 samples (65 males and 15 females) using the DxI® immunoassay (Beckman Coulter).

Results: The calibration curves showed good linearity with a mean R^2 coefficient of 0.9986 (ANOVA P-value: 2.45E-08). The limit of quantification (LOQ) of the method was set at 0.01 ng/mL. Passing Bablok regression analysis showed slopes of 1.07 and 0.58 in males and females, respectively. Bland Altman plots revealed mean differences of -0.01 and -0.11 ng/mL for males and females, respectively. The method showed accurate results according to external quality control samples (UKNEQAS). The chromatographic conditions also showed a clear separation between testosterone, aldosterone, androstenedione, 17-OH-progesterone and 11-deoxycortisol allowing a simultaneous measurement of those analytes.

Conclusion: Our LC-MS/MS method is sensitive enough to measure testosterone levels in female samples with a good accuracy. The bias observed compared to immunoassays is more important in female than in male samples.

P07-12

Pheochromocytoma biochemical diagnosis

Ferreira M (1), Titonel S (2), Rego M (3), Reguengo H (3)

(1) Hospital de Santo António - Centro Hospitalar do Porto, Endocrinology, Diabetes and Metabolism, Porto, Portugal
(2) Hospital de Santo António - Centro Hospitalar do Porto, Clinical Pathology, Porto, Portugal
(3) Hospital de Santo António - Centro Hospitalar do Porto, Clinical Chemistry, Porto, Portugal

Corresponding author: martaffagferreira@hotmail.com

Background: Pheochromocytoma is an adrenal tumor which produces catecholamines. The measurement of catecholamines and/or its metabolites in blood or urine are essential to diagnosis. It isn't clear from which value the diagnosis should be considered. This work compares levels of catecholamines or its metabolites in urine among patients with and without pheochromocytoma.

Materials and methods: Selection of patients with pheochromocytoma diagnosed from January/1990 to December/2011, followed in Centro Hospitalar do Porto, and patients with false positive tests. Demographical, clinical and analytical data were collected and analysed in SPSS 20.0. Results 22 patients had pheochromocytoma (50% were men), with 47.8 ± 16.6 years-old (17-70). Nineteen patients had elevated normetanephrine or metanephrine (the other 3 patients had high levels of norepinephrine, dopamine or VMA). Twenty patients had at least one increased metabolite 3 times above the upper limit (UL) of the reference range (RR). Nineteen patients (86.4%) had at least one increased metabolite 4 times above the UL of the RR. Among patients without pheochromocytoma, sometimes one metabolite was increased (mostly norepinephrine, normetanephrine or VMA), but it was always below 2.5 times the UL of the RR. In 17 (77%) patients, the metabolites levels weren't above 2 times the UL of the RR.

Conclusions: In the authors' laboratory, the pheochromocytoma biochemical diagnosis must be considered when there is an increased metabolite above 4 times the UL of the RR. When the levels are increased just 2 or 2.5 times the upper limit of the RR, it should be excluded causes of false positive results.

P07-13

Comparison of the results of HPLC methods for determination of methanephrine and normethanephrine from urine and blood plasma considering the diagnosis of tumor pheochromocytoma

Brabcová Vránková A, Widimský Jr. J, Zelinka T, Škrha J

3rd Internal Department, First Faculty of Medicine and General Teaching Hospital, Charles University in Prague, Prague, Czech Republic

Corresponding author: alice.vrankova@lf1.cuni.cz

Background: Determination of catecholamines and their O-methyl metabolites normetanephrine (NMN) and metanephrine (MN), in biological fluids plays an important role in the diagnosis of pheochromocytoma (PHEO) - chromaffin cells tumor.

The aim of the study was to compare a HPLC with electrochemical detection method (HPLC-ED) for the determination of methanephrine and normethanephrine in blood plasma and a HPLC with fluorescence detection method (HPLC-FLD) for the determination of the same analytes in conjugated form from urine. Both methods interpret the results in relation to the presence of PHEO. The ability of methods to distinguish the patients with and without PHEO has been proved.

Materials and methods: Determination of metanephrines from plasma (HPLC-ED) and from urine (HPLC-FLD). Standards: NMN ((±) normetanephrine hydrochloride), MN ((±) metanephrine hydrochloride) and HMBA (4-hydroxy-3-methoxybenzylamine-hydrochloride) used as internal standard (Sigma-Aldrich, St.Louis, USA). Chromatography: HPLC system Agilent 1100 (Agilent Technologies, Wilmington, USA). Metanephrines from plasma are determinated in free form, for this reason is possible to directly start by solid phase ex-

traction (SPE) without necessity of hydrolysis. Urine sample is necessary to pass through an acid hydrolysis. During this procedure, releasing of conjugated metanephrines from a bond is happened. Hydrolysis is folloved by SPE. Eluted samples are applied onto a HPLC reversed phase column.

Results: The sensitivity and specificity of methods in tumor diagnosis has been calculated. It has been shown that the sensitivity of both methods has reached 100%, the specificity of methods is lower (94% for the HPLC-ED method and 80% for the HPLC-FLD).

Conclusions: The sensitivity shows excellent ability of both methods to recognize the patients with PHEO. Weaker specificity mainly of the HPLC-FLD method rarely admits false-positive results.

Acknowledgments: Financial support from the project 5302-28002PVK and MPO-Tip FR-TI4/331.

P08 – Evaluation of analytical systems

P08-01

Evaluation of the Sysmex UF-1000i urinanalyzer

Pinter E, Toth J, Konderák J

Synlab Hungary Ltd, Medical Biochemical Department, Budapest, Hungary

Corresponding author: pinter45@gmail.com

Background: The aim of this study was to compare the results of Sysmex UF 1000i analyzer for red blood cells (RBC), white blood cells (WBC), epithel cells (EPI), small round cells (SRC) and pathological cast against manual microscopy of uncentrifuged urine specimens using Fuchs-Rosenthal cell counting chamber.

Materials and methods: Sample size: 500 outpatient urines. Carryover, precision, Passing&Bablok regression, Pearson correlation, Receiver Operating Curves (ROC) and diagnostic accuracy were tested.

Results: Carry over: 0.465% for RBC, 0.117% for WBC 0,19%, for EPI and 0.058% for BACT. Within-run imprecision of cell counts expressed as CV% (mean cell count/µl) was found for RBC 6.86%, for WBC 6.97%, for EPI 35%. Between run imprecision was carried out in 30 replicates of two urine control at different concentrations for RBC, WBC, EPI, BACT, and CAST. The mean and CV% was calculated. Passing-Bablok regression were determined for RBC: y = -0.0321 + 1.0383x, for WBC: y = -0.2990 + 1.0499x, for EPI y = 0.2285 + 1.0191x, for SRC y = -0.2161 + 1.6129x, for CAST y = 0.0 + 4.2666x, respectively. Diagnostic accuracy of Sysmex UF1000i showed the following results: 93.2% for RBC, 97.2% for WBC, 92.6% for EPI, 97% for SRC and 60.0% for PC. (Further results: Sensitivity data are: RBC: 96.4%, WBC: 98.0%, EPI: 96.7%, SRC: 60.0%, PC: 79.6%. Specificity data are: RBC: 90.5%, WBC: 95.1%, EPI: 89.7%, SRC: 98.2%, PC: 56.9%.)

Conclusion: Sysmex UF 1000i urine analyzer eliminated manual sample preparation. It has proven good precision for analyzing cellular elements.

P08-02

Evaluating performance of Cobas c311 and Cobas c501 in the emergency laboratory setting

Lapić I, Lukić I, Kutnjak V, Fressl Juroš G, Rogić D

Clinical Hospital Center Zagreb, Clinical Institute of Laboratory Diagnostics, Zagreb, Croatia

Corresponding author: ivana.lapic@hotmail.com

Background: The aim of our study was to perform the analytical validation of the automated biochemistry analyzers Cobas c311 and c501 (Roche Diagnostics, Germany), for the purposes of the emergency laboratory.

Materials and methods: Validation included assessment of within-day (N = 30) and between-day imprecision (N = 30), inaccuracy (bias) (N = 30), linearity and method comparison with the hitherto used analyzer Olympus AU2700 (N = 30). Statistical

analysis was performed and the results were judged by comparing with the specifications according to Westgard.

Results: Statistical analysis fulfilled almost all required criteria. Within-day and between-day imprecision, evaluated at two levels, for all analytes on both analyzers revealed acceptable coefficients of variation (CV < 5%). Bias for calcium, glucose, total proteins and urea on Cobas c311 was beyond the range of desirable specifications while on Cobas c501 only bias for chlorides exceeded the recommended value. For all analytes, the range of linearity defined by the manufacturer was proved. According to the Passing-Bablok regression analysis, the results of the comparison study showed statistically negligible deviations except for direct bilirubin and CRP for which proportional error was identified. Moreover, method comparison yielded high coefficients of correlation (r > 0.95) for all analytes determined on both analyzers.

Conclusion: Cobas analyzers show acceptable precision and accuracy for all but a few analytes for which slight adjustment of the instrument factor is required, meet the established requirements and specifications and therefore can be implemented in routine laboratory work. However, the performance of Cobas c311 does not meet the turnaround time for the high throughput emergency laboratory requirements.

P08-03

Analytical performance of the OC-Sensor DIANA immunochemical faecal occult blood test

Halloran S, Pearson S, Piggott C, Young M

NHS Bowel Cancer Screening Programme, Southern Hub, Guildford, United Kingdom

Corresponding author: s.halloran@nhs.net

Background: The automated analyzer, OC-Sensor DIANA (Eiken Chemical Co., Ltd., Japan), will be used in the 'FIT for Follow-Up' study. The study will investigate the three-year programme sensitivity of an annual faecal immunochemical test (FIT) for haemoglobin (Hb) for the detection of advanced adenomas or colorectal cancer in patients diagnosed with intermediate risk polyps through the NHS Bowel Cancer Screening Programme.

Materials and methods: The analytical performance of DIANA was evaluated using buffered Hb solutions and compared with that reported in 2009.

Results: The Clinical and Laboratory Standards Institute (CLSI) five-day imprecision protocol demonstrated good within-assay and total imprecision at concentrations > 159 ng Hb/mL buffer (CV < 1.7% and < 3.4% respectively). Within-assay and total imprecision at 43 ng/mL was poor (CV 11.5%). The manufacturers do not recommend the use of quantitative results < 50 ng/mL. The QC materials containing average concentrations of 84, 152 and 658 ng/mL gave within-assay CVs of < 1.3% and for control material with average concentrations of 153 and 655 ng/mL it gave day-to-day CVs of < 2.6%. The assay was linear between 45 and 930 ng/mL supporting the manufacturer's claimed measuring range of 50–1000 ng/mL. The regression equations from 2011 (y = 1.03x-4.99) and 2009 (y = 1.06x+8.54) were similar. The analyzer's sample carry-over effect in both evaluations was well within acceptable limits for interaction at < 0.5%.

Conclusion: Analyzer performance was consistent with manufacturer's claims. 'Baseline' performance will be compared with future evaluations performed at regular intervals throughout the course of the 'FIT for Follow-Up' study.

P08-04

Evaluation and validation of the Cobas 6000 analyzer series modules c501: the urine analytes

Šimičević L, Cigula Kurajica V, Vogrinc Ž, Sertić J

Clinical Hospital Center Zagreb, Clinical Institute of Laboratory Diagnosis, Zagreb, Croatia

Corresponding author: zvogrinc@kbc-zagreb.hr

Background: The aim of study was to assess the analytical performance of the Cobas 6000 analyzer series modules c501 (Roche Diagnostics) and to validate it in our routine set of urine analytes.

Materials and methods: We investigated 12 routine urine analytes tests (amylase, glucose, urea, creatinine, urate, total proteins, sodium, potassium, chloride, calcium, phosphate, magnesium) on two modules c501 (R1+R2). The evaluation protocol consisted of imprecision: within-run (10 sequential runs) and between-run (10 consecutive working days, 2 sequential runs) with commercial controls (Liquicheck, Biorad; Precinorm PUC/Precipath PUC, Roche), inaccuracy (N = 20), and method comparison (routine urine samples, N = 30) vs. Beckman Coulter AU640.

Results: All analytes on both modules have within-run imprecision < 3%, except phosphate and magnesium (R2). For all analytes between-run imprecision was < 5%. All analytes fulfilled quality requirements for imprecision and for total error. A quality requirement for inaccuracy was met by all analytes on both modules with exception of urate on R1 and amylase on R1+R2. The correlation with comparison method showed no difference between methods for glucose, amylase, urea, sodium, phosphate, magnesium on R2 and for potassium, amylase, urea on R1. Constant difference was observed for five analytes on R1, and three on R2. Proportional difference was found for five analytes on R1, and two on R2. Two tests need further investigation, chloride on R1, and creatinine on both modules, due to significant deviation from linearity.

Conclusions: Cobas 6000 analyzer showed optimal analytical performance with mostly fulfilled quality control requirements and acceptable method comparisons for urine analytes.

P08-05

Differences between bromcresol green and bromcresol purple measured albumin

Garcia Moreira V, Martinez Gago M, Fernandez Leivas A, Bachiller Sister M, Laborda Gonzalez B, Fernandez Rodriguez E

Hospital de Cabueñes, Servicio de Analisis Clinicos, Gijon, Spain

Corresponding author: vgmoreira@yahoo.es

Background: Usually, albumin is measured by techniques employing dye-binding assays: bromcresol green (BCG) and bromcresol purple (BCP). In management of an individual patient with albumin results from different laboratories, the appreciation of the methodology used and the ability to convert results between methods will be helpful.

Material and methods: 85 plasma and 80 serum samples were analyzed by BCP(DimensionRX- Siemens) and BCG (Advia2400-Siemens) methods. Of these, 75 serum samples were also run on capillary zone electrophoresis (CZE). Significant differences were determined by Student paired-t and ANOVA tests, using MedCalc. Significance was set at $P < 0.05$. Passing-Bablok regression, Bland-Altman plots and Pearson correlation were used to examine the relationship between methods.

Results: Correlation AlbBCG and AlbBCP was $r = 0.948$ ($P < 0.01$), but the mean difference was large 6.42g/L (CI 95%mean: 5.88-6.96) ($P < 0.01$). The regression equation was AlbBCG = 0.784 AlbBCP + 12.58. Bland-Altman plots shows greater bias at lower albumin concentrations. For the normal concentration group (G1; AlbBCP ≥ 35 g/L), mild hypoalbuminemia (G2; 30-35 g/L), moderate hypoalbuminemia (G3; 20-29 g/L) and severe hypoalbuminemia (G4; ≤ 20 g/L), mean differences were 3.07 g/L, 6.33 g/L, 7.50 g/L and 9.92 g/L respectively (ANOVA; $P < 0.01$). Using the Newman-Keuls

post-hoc test, differences were found between G1 *vs*. G2, G3 and G4 as well as between G4 versus G2 and G3 ($P < 0.05$). Also, a positive bias was observed between BCG/CZE (mean 3.54 g/L) and good correlation between CZE/BCP with a mean difference < 1g/L.

Conclusion: Albumin results from BCP and BCG methods may result in unacceptable differences and clinical confusion. The BCP method is superior method to evaluate the serum albumin levels, due to BCP albumin is more specific than BCG.

P08-06

Sodium measurements in urine by the patient at home: primary limitations overcome

Schenk P, Romijn F, Kolb R, Cobbaert C

Leiden University Medical Center, Department of Clinical Chemistry, Leiden, Netherlands

Corresponding author: p.w.schenk@lumc.nl

Background: It might be useful if hypertensive patients can self-monitor their sodium intake by assessing sodium excretion in urine at home.

Materials and methods: We evaluated a novel system, consisting of disposable syringes + LabChips and the Medimate Multireader, intended for POC sodium measurements in human urine, using moving boundary and capillary zone electrophoresis and conductivity detection. The system was compared to the Roche Modular ISE (standard laboratory method) and IL 943 flame photometer (gold standard), and precision was determined. Ease of use was evaluated by two experienced laboratory technicians.

Results: We compared the Multireader to both laboratory methods for 38 different 24-hour urine specimens (range: 33-195 mmol/L): > 5% of results were outside the allowable total error interval (+/-28.8%). Multireader CVs were 6.6% and 16.4% at a level of 156 and 52 mmol/L sodium, respectively (with CVs in the 1-2% and 0.1-0.2% range for ISE and flame photometry, respectively). Accordingly, the software algorithm converting electropherograms to sodium concentrations was adapted. The Multireader was then compared to flame photometry in a set of new experiments: none of 14 results (range: 70-302 mmol/L) showed significant bias. Multireader CVs were 2.2% and 3.1% at a level of 108 and 313 mmol/L, respectively. The new algorithm therefore shows an evident improvement in analytical performance. Despite several innovations, ease of use remained underdeveloped.

Conclusions: Although analytical CVs are considerably higher, the POC method seems clinically equivalent to flame photometry. The novel system is definitely promising, but its ease of use has to be improved.

P08-07

Evaluation and validation of the Cobas 6000 analyzer modules c501: the routine serum analytes

Šimičević L, Cigula Kurajica V, Vogrinc Ž, Sertić J

Clinical Hospital Center Zagreb, Clinical Institute of Laboratory Diagnostics, Zagreb, Croatia

Corresponding author: zvogrinc@kbc-zagreb.hr

Background: The aim of the study was to assess the analytical performance of the Cobas 6000 analyzer series modules c501 (Roche Diagnostics) and to validate it in routine set of serum analytes.

Materials and methods: We investigated complete routine serum tests for 36 analytes and preliminary present the results for cholesterol, HDL, LDL, triglycerides, uric acid, iron, and UIBC on two modules c501 (R1+R2) plus ACE (R1), and cooper (R2). The evaluation protocol consisted of imprecision: within-run (10 sequential runs) and between-run (10 consecutive working days, 2 sequential runs) with commercial controls (PreciControl ClinChem Multi 1/2, Roche; HumAsy Control 2/3, Randox; ACE controls N/H, Bühlmann), inaccuracy

(N = 20), and method comparison (routine serum samples, N = 30) *vs*. Beckman Coulter AU640.

Results: The majority of analytes on the assigned modules have a within-run imprecision < 2%. For all analytes, except cooper and UIBC, the between-run imprecision was < 3%. Quality requirements for imprecision and total error were met by all analytes on the assigned modules, excluding cooper. Quality requirement for inaccuracy were fulfilled by all analytes with the exception of HDL (R2). The correlation with comparison method showed no difference between methods for cholesterol, LDL, cooper, uric acid, and ACE on the assigned modules. Triglycerides and HDL showed some difference on both modules. UIBC and triglycerides on R1, due to significant deviation from linearity need further investigation.

Conclusions: These preliminary results showed that Cobas 6000 analyzer has optimal analytical performance with mostly fulfilled quality control requirements and acceptable method comparisons for our routine serum analytes.

P08-08

Normalized MEDx chart – useful tool for quick assessment of multiple method performance

Šupak Smolčić V (1), Bilić-Zulle L (1,2)

(1) Rijeka Clinical Hospital Center, Clinical Institute of Laboratory Diagnostics, Rijeka, Croatia
(2) Rijeka University School of Medicine, Department of Medical Informatics, Rijeka, Croatia

Corresponding author: vesnasupak@gmail.com

Background: Method validation includes determination of inaccuracy (bias) and imprecision (CV) which represent systemic and random error respectively, and which are basic parameters for calculation of total error (TE). Each method is evaluated regarding its recommended quality specification. Aim of this presentation is to introduce normalized MEDx chart, a simple graphical method for quick assessment of analytical method performance when multiple methods are validated simultaneously.

Materials and methods: Data were collected from method validation study performed on Roche Cobas 6000 analyzer c501 (Roche, Germany) (published in *Biochemia Medica* 2011;21(2):182-90). TE is calculated according to equation: TE = Bias + σCV where σ stands for desired confidence level (σ=2-6). This equation is the basis for MEDx chart where each method is presented as operating point defined by Bias on y-axis and CV on x-axis. Several criteria for σ value are presented on the chart according to which method performance is classified. If imprecision and inaccuracy are expressed as percentage of TE and such adjustment of x and y axis scaling is made than all operating points can be presented on the same chart called normalized MEDx chart.

Results: Validation of 30 analytical methods performance at Roche Cobas 6000 biochemistry analyzer are presented graphically as normalized MEDx chart.

Conclusion: Normalized MEDx chart is useful tool for comprehensive presentation of validation data and quick overall judgment of analyzer performance according to recommended quality specifications. Chart is easy to create and even easier to interpret as opposed to comparing numerous figures and analyzing large tables.

P08-09

Evaluation of haematological analyzer Cell Dyn Ruby

Šega M, Perbil Lazič K, Lokar L

University Clinical Centre Maribor, Centre of Transfusion Medicine, Maribor, Slovenia

Corresponding author: sega.marija@gmail.com

Background: The goal of evaluation was to estimate the working of new haematological analyzer CELL-DYN Ruby. The aim of measurements of parameters WBC, RBC, HGB, HCT, MCV, MCH, and PLT

as recommended by CLSI was to determine the "Long-term imprecision" and "bias" and provide an assessment of the acceptability of a new analyzer.

Materials and methods: We have used commercial controls to evaluate „Long-term imprecision'' and determined standard deviation and coefficient of variation through statistical analysis of the results. To compare two methods the testing was performed on the extinguishers Cell Dyn 1700 and Cell Dyn Ruby within three hours. Every day the testing of series 8-10 samples was performed in the sequence 1,2,3,4,5,6,7,8 and duplicates in the sequence 8,7,6,5,4,3,2,1 ever. Using statistical analysis, we have compared the comparability of methods and calculated power connections with a correlation factor.

Results: In estimating analytical variability, we have determined the maximum coefficient of variation of platelets from 2% to 5%. In assessing the comparison of methods Cell Dyn 1700 and Cell Dyn Ruby, we have found out that both methods are comparable. Correlation coefficients for WBC, RBC, HGB, HCT and PLT were between 0.9773 and 0.9976, for MCV and MCH they were between 0.9476 and 0.9988.

Conclusions: Based on our results, we can conclude that the haematological analyzer Cell Dyn Ruby is suitable for determination of complete blood counts in the hemostasiological laboratory.

P08-10

Overestimation of albumin (bromcresol green method): influence of acute-phase serum globulins

Garcia Moreira V, Martinez Gago M, Laborda Gonzalez B, Fernandez Leivas A, Llorente Torres A, Fernandez Rodriguez E

Hospital de Cabueñes, Servicio de Analisis Clinicos, Gijon, Spain

Corresponding author: eloyfr@gmail.com

Background: As the most specific method for albumin measurement (immunonephelometry) is expensive, most laboratories use dye-binding methods, being the two most common the bromcresol green (AlbBCG) and bromcresol purple (AlbBCP). AlbBCG and AlbBCP often yield discordant results, with a mean difference about 5 g/L. The objective of this study was to evaluate the influence of serum globulin on discrepancies albumin results measuring by BCG and BCP methods.

Material and methods: Concentrations of serum albumin, globulins (alpha1-, alpha2-, beta and gamma) and C-Reactive Protein (CRP) were analyzed in 75 serum specimens by BCG and BCP and capillary zone electrophoresis (CZE) and nephelometry respectively. Comparisons were performed using Student t-test for paired data, Pearson correlation and Bland-Altman plot, using MedCalc software. Significance was set at $P < 0.05$.

Results: Correlation between AlbBCP-AlbBCG was 0.976 ($P < 0.01$) but the mean difference was 4.53g/L. Between AlbBCG- AlbCZE mean difference was 3.54g/L. AlbBCP is in good agreement AlbCZE estimation with a mean difference of 0.97g/L. Differences between AlbBCG and AlbBCP become more evident with lower albumin concentration. AlbBCG assay bias shown a good correlation with alpha1-globulin concentrations ($r = 0.746$; $P < 0.01$); a moderate ($r = 0.633$; $P < 0.01$) and weak correlation ($r = 0.575$; $P < 0.01$) was observed with CRP and alpha2-globulin, respectively; finally, we found no correlation with beta-globulin ($r = 0.151$; $P = 0.228$), and a poor correlation with gamma-globulin ($r = -0.272$; $P = 0.02$).

Conclusion: BCG method is not absolutely specific for albumin. Serum acute phase globulins (alpha1 and alpha2) contribute to the overestimation of albumin concentration by BCG assay, with the greatest effects observed for alpha1-globulin.

P08-11

Comparison of sodium values between emergency and routine biochemical laboratory

Fernandez Leivas A, Garcia Moreira V, Martinez Gago M, Marcos Corona L, Garcia Alonso S, Fernandez Rodriguez E

Hospital de Cabueñes, Servicio de Analisis Clinicos, Gijon, Spain

Corresponding author: anafleivas@hotmail.com

Introduction: In our hospital, the determination of ions is performed by indirect potentiometry: Dimension RxL-Max (Siemens) at the emergency laboratory and Advia2400(Siemens) in the routine laboratory. Suspecting a downward trend in the determination of sodium(Na) in the ER laboratory, we process the same samples on both analyzers, using the Advia2400 as a reference method.

Material and methods: On 3 different days, 158 serum samples were processed in parallel in both analyzers. Statistical analysis of the results was performed using MedCalc® software, using the Kolmogorov-Smirnov test, Pearson correlation and linear regression. Statistical significance was set at $P<0.05$.

Results: The overall correlation between the two methods was good, using Pearson's coefficient of correlation tes, $r = 0.894$ (95 CI% = 0.858-0.922) ($P < 0.01$).We calculated a regression equation to transform the results of Dimension in Advia values:y (Advia) = 9.6773 + 0.9356x (Dimension). Samples were classified into 2 groups according Advia results:hyponatremia (< 135 mEq/L) and not hyponatremia(> 135 mEq/L). After this, the hyponatremic cases(N = 12) were subdivided into three groups according to the degree of discrepancy between methods: 25% mild (difference ± 1 mEq/L), 42% moderate(± 2 mEq/L) and 33% severe (± 3 mEq/L). We obtained a 13% false negatives (Advia Na < 135 mEq/L and normal in Dimension > 135 mEq/L) and 9% false positives (Advia normal and low in Dimension). Applying the previously calculated regression equation to convert the Dimension results on Advia ones, these percentages were changed to a 26.1% of false negatives and 3% of false positives.

Conclusions: Contrary to the initial suspicion, we found more false negatives than false positives, 13% and 8.9% respectively. So, the application of the linear regression line, while reducing the false positive rate (3%), doubles the number of false negatives (26.1%). The linear regression increased by 7.1% the total number of differing values, there by not suggest their application in clinical practice.

P08-12

Analytical evaluation of the new random access bench top analyser RX daytona plus

Brown M, McGivern P, O'Neill G, Campbell J, Fitzgerald S

Randox Laboratories Limited, Research and Development, Crumlin, United Kingdom

Corresponding author: scientific.publications@randox.com

Background: The availability of fully automated clinical chemistry analyzers capable of facilitating quick and precise analysis is beneficial in the process of patient diagnosis. This is relevant to those assays used, for example, in the diagnosis of a cardiac event. This study reports the evaluation of a newly developed fully automated bench top system, the RX daytona plus. This is demonstrated with the evaluation of an aspartate aminotransferase (AST) assay. Elevated levels of AST can signal myocardial infarction.

Materials and methods: On-board and calibration stabilities were tested by storing the reagents uncapped on the analyzer for 28 days. Assay precision was assessed by testing serum samples at defined levels, 2 replicates twice a day for 20 days. Correlation studies were conducted using another commercially available clinical chemistry analyzer.

Results: The AST reagent presents an on-board stability and calibration frequency of 28 days. The assay was found to have a Limit of Blank of 0.5 U/L and be linear up to 927 U/L. The within-run and total precision for three different concentration

levels typically had %CVs of ≤ 3.5%. In the correlation study 60 serum patient samples were tested and the following linear regression equation was achieved *vs*. analyser A: Y = 1.03x + 4.64; r = 0.999.

Conclusions: The results from this evaluation of the AST assay on the new bench top RX daytona plus analyzer indicate optimal analytical performance and overall the system represents a useful cost-effective analytical tool for the clinical chemistry laboratory.

P08-13

The performance of Cobas c311 in stat laboratory

Kutnjak V (1), Rogić D (2), Fressl Juroš G (2)

(1) University Hospital Center Zagreb, Clinical Department of Transfusion Medicine and Transplantation Biology, Zagreb, Croatia
(2) University Hospital Center Zagreb, Clinical Institute of Laboratory Diagnostics, Zagreb, Croatia

Corresponding author: kutnjakvl@gmail.com

Background: The objective of this study was to perform the analytical evaluation of the biochemistry analyzer Roche Cobas c311 and compare TAT of emergency tests with biochemistry analyzer Olympus AU 480. Short validation of the Cobas c311 was performed according the guidelines of the European Committee for Laboratory standards.

Materials and methods: The tested analytes in this study were : glucose, urea, creatinine, total and direct bilirubin, alpha-amylase, alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase, lactat dehidrogenase, gama-glutamyltransferase, creatine kinase , lipase, C-reactive protein, calcium, sodium, potassium and chloride. Research included determination of within-run and between–run imprecision, inaccuracy, calculated total error and method comparison with Olympus AU 480. In addition, we compared tournaround time (TAT).

Results: Coefficients of variation for within-run imprecision for all tested analytes were below 5%. Coefficients of variation for between-day imprecision were compared to quality specifications. CVs for calcium, sodium and chloride are higher than recommended. Results for total inaccuracy (bias) revealed that urea, calcium, sodium, potassium and chloride have higher total inaccuracy than recommended. In addition, calcium, sodium and chloride are not in accordance with recommended specification when comparing total error with quality recommendations (results are higher than recommended values). Coefficients of correlation for majority of analytes are r > 0.98 except for calcium (r = 0.884), sodium (r = 0.837) and chloride (r = 0.881). TAT for the Cobas c311 was on the average 11% longer then Olympus 480.

Conclusion: Cobas c311 biochemistry analyzer is acceptable for emergency purposes, however the overall TAT is slightly longer than recommended.

P08-14

Comparison of Emerald and CELL-DYN 1800 for white blood cells and granulocytes in oncology patients

Mittel K, Supak Smolcic V, Dvornik S

Clinical Hospital Center Rijeka, Rijeka, Croatia, Clinical Department of Laboratory Diagnostics, Rijeka, Croatia

Corresponding author: koraljka.mittel@lycos.com

Background: The absolute number of the white blood cells (WBC) and neutrophil granulocytes (GRAN) in peripheral blood of patients undergoing chemotherapy or radiotherapy is an important indicator of their immunodeficiency. Capillary blood sampling from finger is an option in case of frequent blood controls and damaged blood vessels. Hematology analyzers Abbot CELL-DYN Emerald and CELL-DYN 1800 are good choices because of the small sample volume needed for analyses (9.8 and 30 uL respectively). The aim of the study was to compare the performance of

CELL-DYN Emerald and CELL-DYN 1800 for WBC and GRAN measurement.

Materials and methods: Capillary blood samples of 193 patients were analyzed on CELL-DYN Emerald and CELL-DYN 1800 for WBC and GRAN. Blood was collected from finger using BD Microtainer® tube with K2-EDTA anticoagulant. Passing-Bablok regression was used to compare results from two analyzers.

Results: Correlation coefficient for both parameters is r = 0.98, P < 0.001. Intercept for WBC is 0.31, 95%CI 0.15-0.48 and slope is 0.94, 95%CI 0.92-0.97. For GRAN intercept is 0.07, 95%CI -0.01-0.13 and slope is 0.94, 95%CI 0.92-0.96. Results indicated small constant and proportional error for WBC and small proportional error for GRAN.

Conclusions: Despite the small constant and proportional error for WBC and small proportional error for GRAN, both analyzers can be used to access WBC and GRAN count in capillary blood samples. CELL-DYN Emerald needs very small sample volume for analysis and thus it is very useful for capillary blood samples from oncology patients.

P08-15

Body fluids automated cell count: a comparison study between Sysmex XE-5000 and Siemens ADVIA 2120i

Rego M, Titonel S

Centro Hospitalar do Porto, Serviço de Química Clínica, Porto, Portugal

Corresponding author: martacostarego@sapo.pt

Introduction: Body fluid cytology, including total cell count and differentiation, continues to be a time-consuming task in modern clinical laboratories. The evolution of hematological analyzers, with new approaches to the counting methodology, has brought new possibilities in biological fluids cytology. The aim of this study is to assess whether Sysmex XE-5000 (Sysmex Corporation, Kobe, Japan) and Siemens ADVIA 2120i (Siemens Healthcare Diagnostics Inc., New York, USA) hematology analyzers can replace the manual reference method in daily clinical onset.

Materials and methods: One hundred thirty three (N = 133) non-CSF body fluid samples were prospectively studied, including 74 ascitic fluids (55.6%), 49 pleural fluids (36.8%), 3 pericardial fluids (2.3%), 4 synovial fluids (3.0%) and 3 drainage liquids (2.3%). Each fluid was analyzed by two experienced cytologists and the two above mentioned hematology analyzers.

Results and conclusions: Our results indicate that in serous fluids – ascitic, pleural and pericardial – is possible to use either Sysmex XE-5000 or Siemens ADVIA 2120i to perform total cell count. However, cell differentiation using these approaches lacks both enough sensibility and specificity and therefore they should not be used. Finally, the automated analysis did not provide accurate results for other body fluids.

P09 – Haematology 1

P09-01

Verification of CD34+ stem cell analysis according to ISHAGE protocol on Beckton Dickinson FACSCanto

Sokolić I, Šiftar Z, Kardum Paro M, Flegar Meštrić Z

C.H."Merkur", Department of Clinical Chemistry and Laboratory Medicine, Zagreb, Croatia

Corresponding author: ivica.sokolic@gmail.com

Background: Implementation of new analytical equipment into routine work requires verification procedure. We have verified flow cytometer Beckton Dickinson FACSCanto II for ISHAGE protocol (Sutherland et al, 1996, J Hematotherapy, 5:213-226) stem cell (CD34+) analysis according to CLSI EP-A2-User Verification of Performance for Precision and Trueness; Approved Guideline-2nd ed.,Vol.25,No.17.,2005. in concordance with ISO 15189.

Materials and methods: Analysis was performed on peripheral blood samples, fresh leukapheresates of peripheral blood and BC StemCell Control Kit samples. Acquisition data were analyzed using BD FACSDiva 6.0 software. Verification included assessment of random error check based upon triplicate measurement of commercial samples five days in a row, assessment of measurement uncertainty, conformation of linearity, comparison of results from reference analyzer and analysis of external quality control samples.

Results: Performance characteristics: within-laboratory precision for Stem Cell Low (11.6 x 10^6 / L) was 9.2%, for Stem Cell High (31.5 x 10^6 /L) 8.0%. We confirmed linearity on peripheral blood sample 0-97.2 x 10^6 /L. Measurement uncertainty for Stem Cell Low was 28.4% and Stem Cell High 19.2%. Comparison of results with Beckman Coulter Cytomix FC500 was satisfactory (Passing Bablock, Y = -0.01117 + 1.0112x, P > 0.10). UK NEQAS for Immunophenotyping, Stem Cell enumeration scheme results were within target values (median ± 25 percentile) for CD34+ absolute and relative count.

Conclusion: According to our verification procedure, BD FACS Canto II is cytometer of choice for performing CD 34+ stem cell absolute count analysis.

P09-02

Copper deficiencies simulating a myelodysplastic syndrome

Gallego A (1), Vera J (2), Peral E (3), Martín J (1), Bermudo C (1), Fábregas M (2)

(1) Hospital Universitario Virgen Macarena, Bioquímica Clínica, Sevilla, Spain
(2) Hospital Universitario Virgen Macarena, Hematología, Sevilla, Spain
(3) Hospital Universitario Virgen Macarena, Medicina Interna, Sevilla, Spain

Corresponding author: gallego.am@gmail.com

Introduction: Copper is an essential trace element present in a number of metalloproteins and appears to be required for the absorption and utilization of iron. Copper deficiency has been described in malnourished children, in both infants and adults receiving prolonged enteral or parenteral alimentation, and following gastric resection or bariatric gastric reduction surgery. It is already known that cupper deficiency could resemble a myelodysplastic syndrome with anemia, neutropenia and trombocytopenia.

Case report: Patient 32 years male with spastic tetraplegia after severe TBI caused by a traffic accident in 2009. It was decided to place jejunostomy in June 2010. In June 2011 a normocytic anemia was detected without signs of bleeding according both clinically and by the diagnostic tests performed and treated according to protocol. In January 2012 was readmitted for persistent leucopenia and anemia in transfusion range. It was performed bone marrow aspirate, being found a bicytopenia, dysplastic features, reactive bone and good representation of the three series. Values copper and ceruloplasmin levels were requested, being respectively: 36 mcg/dL (Normal being 65-165) and 12 mg/dL (Normal 20-40). Parenteral alimentation was maintained with supplements of copper, recovering values cupperhemic to enter the normal range and resolving the bicitopeny and symptoms secondary to it.

Conclusion: In patients with unexplained refractory cytopenias, dysplastic features and comorbidities that may trigger malnutrition such as parenteral alimentation, you should order determination of copper and ceruloplasmin levels before diagnosis of myelodysplastic syndrome.

P09-03

Multiple myeloma: a case report

Cachapuz I (1), Branco A (2), Paiva A (2), Magalhaes C (1), Costa M (2)

(1) Matosinhos Local Health Unit , Clinical Pathology Service, Oporto, Portugal
(2) Matosinhos Local Health Unit, Nephrology Service, Oporto, Portugal

Corresponding author: cachapuz.isabel@gmail.com

Introduction: The multiple myeloma is a neoplasm of B cells, associated to the presence of a monoclonal protein in serum and/or urine. It is clinically manifested by different symptoms.

Case report: 58 year-old woman, with depression, obesity and hypertension, presented at the ER (Emergency Room) of Pedro Hispano Hospital on the 13th January 2012 due to nonoliguric acute kidney injury (creatinine: 7.7 mg/dL), anemia (Hb: 7.1 mg/dL),calcium 8.2 mg/dL, proteinuria (7 g/dia). Renal echography: normal. Blood transfusion was made. Declared having anemia for 6 months, under study, and she had already made an upper digestive endoscopic and colonoscopy both without changes. Three months before hospitalization: 10 kg loss, asthenia, anorexia, nausea, pain in the left costal margin. At the ER, abdominal CT scan revealed lytic lesions. The day after, the patient made a serum proteinogram: beta peak. The serum protein electrophoresis (SPE) revealed the presence of a monoclonal component lambda (4.54 g/L). Since there was no recovery of kidney function, haemodialysis was started. A myelogram was performed and revealed pathological plasma cells. The cytogenetic laboratory didn't detect IGH_MMSET fusion, nor 17p13 deletion (gene TP53), at the bone marrow. She started chemotherapy with Bortezomib and Dexametazona on the 20th January 2012, and was treated with high cutoff filters without success in the recovery of kidney function.

Conclusion: This clinical case underlines the importance of the clinical-laboratory interaction for the correct and timely diagnosis of haemato-oncological pathologies. SPE is still an important auxiliary diagnostic tool.

P09-04

Which value can be used as a positivity threshold for Large Unstained Cells on Advia to make slides?

László K, Patonai Z, Kellner V, Fekete M

Synlab Hungary Ltd., Laboratory of Székesfehérvár, Székesfehérvár, Hungary

Corresponding author: laszloki@yahoo.com

Introduction: The Advia 2120i hematological system uses peroxidase staining for white blood cells differential testing. Peroxidase-negative cells, large lymphocytes, plasma cells, blasts are counted to large unstained cells (LUC) category. The manufacturer recommends the normal range for LUC < 4%. If LUC > 4%, it is suggested to make a slide and analyse morphology of cells by microscopy. The aim of this study was to find a real positivity threshold for LUC, where the results by microscopy give additional information to the clinician beyond the mere numerical data.

Materials and methods: ROC curve analysis was performed to obtain the appropriate results. Blood samples of 350 patients were examined by automated Advia 2120i and microscopy. Three analyses were performed and results were considered to be positive as follows, "1": if blasts or any number of reactive/granulated lymphocytes were found by microscopy; "2": if blasts or a few (4-6) or more reactive/granulated lymphocytes-, and "3": if blasts or several (> 7) reactive/granulated lymphocytes were counted by microscopy.

Results: In analysis "1", the positivity threshold for LUC > 4% was observed, the true negative rate (TNR; specificity): 74.76%; the true positive rate (TPR, sensitivity): 88.81%. High number of false positive results (FPR) were detected: 25.24%. In case of "2" the positivity threshold for LUC > 5% was found, TNR: 83.27%, TPR: 66.25%, FPR: 16.73%. In analysis "3" the positivity threshold for LUC > 6%, TNR: 94.08%, TPR: 56.45% and FPR: 5.92% were obtained.

Conclusion: Overall, even LUC > 6% was found to be reliable positivity threshold to request blood smears.

P09-05

Role of P-selectin glycoprotein ligand-1 (PSGL-1) during G-CSF treatment in a mouse model

Miszti Blasius K (1), Felszeghy S (2), Hevessy Z (1), Kappelmayer J (1)

(1) Medical and Health Science Centre, University of Debrecen, Department of Laboratory Medicine, Debrecen, Hungary
(2) Medical and Health Science Centre, University of Debrecen, Department of Anatomy, Debrecen, Hungary

Corresponding author: cornelius@dote.hu

Background: The effect of G-CSF (Neupogen) was investigated in wild-type (WT) and PSGL-1 knock-out (KO) mice to establish the role of this mucin in myeloid cell mobilization. G-CSF activates tissue proteases that cleave several adhesion molecules, thus enhances the mobilization of myeloid cells and haemopoietic stem cells.

Materials and methods: WT and KO mice (12-16 week old, 20-25 gram body weight, N = 15) were treated with a single dose of 250 mg/kg cyclophosphamide to induce cytopenia, subsequently mice received 7.8 µg/kg G-CSF twice a day for 4 days. Retro-orbital blood samples were drawn to determine leukocyte counts by Siemens Advia 120 analyser.

Results: Neutrophil granulocyte count increased upon completion of G-CSF treatment but were significantly different in WT and KO mice (28.3 G/L *vs*. 47.7 G/L), while the monocyte counts were 2.0 G/L and 4.1 G/L. Four days after the last G-CSF treatment, both strains displayed considerably reduced neutrophil (1.8 G/L and 9.8 G/L) and monocyte (0.4 G/L and 1.6 G/L) counts, the values always being higher in KO animals. Contrary, eosinophil granulocyte values became elevated only at this sampling time, being 0.5 G/L (WT) and 1.2 G/L (KO). There was a similar difference in atypical cell counts always being higher in KO animals.

Conclusions: The lack of PSGL-1 results in higher mature and precursor myeloid cell release after G-CSF treatment with delayed effects on eosinophils. The differences are caused by both faster mobilization from the bone marrow and delayed extravasation in the peripheral vessels in PSGL-1-/- animals.

P09-06

Usefulness of red cell indices in differentiating microcitic anemias

Pavela J, Fijačko M, Dobrošević B, Paradinović K, Šerić V

Clinical Hospital Centre Osijek, Department of Clinical Laboratory Diagnostics, Osijek, Croatia

Corresponding author: pavela.jasna@kbo.hr

Backgound: Iron deficiency anemia (IDA) and beta-thalassemia trait (β-TT) are the most common forms of microcytic anemia. This study was conducted to compare the validity of various discrimination indices in differentiating β-TT from IDA by calculating their sensitivity, specificity and Youden's index.

Materials and methods: A total of 146 patients (69 IDA and 77 β-TT) with microcytic anemia were involved in this study. We calculate six discrimination indices i.e. RBC count, Mentzer indeks (MI), Shine & Lal (S&L), Srivastava (SI), England & Fraser (E&F) and Red cell Distribution Width Indeks (RDWI). The number of correctly identified cases were determined and sensitivity, specifity, positive and negative predictive value and Youden's index of each discrimination index were calculated.

Results: The percentage of correctly diagnosed is highest for E&F and RDWI (86%) folowed by MI (79%) index. None of the discrimination index showed 100% sensitivity and specificity. Youden's index wich includes both, sensitivity and specificity is in descending order RDWI > E&F >MI > RBC >SL >SI.

Conclusion: RDWI, England-Fraser and Mentzer index are the most reliable formulae in discrimination between iron deficiency anemia and beta-thalassemia trait.

P09-07

A method of preparing fresh blood samples for Croatian haematology external quality assessment

Nazor A (1), Flegar-Mestric Z (1), Hećimović A (2), Šiftar Z (1), Hundrić Hašpl Ž (2)

(1) University Hospital Merkur, Institute of Clinical Chemistry and Laboratory Medicine, Zagreb, Croatia
(2) Croatian Institute for Transfusion Medicine, Blood bank, Zagreb, Croatia

Corresponding author: zlata.mestric@gmail.com

Background: Croatian EQA involves participation in the National External Quality Assurance Scheme organized under the auspices of the Croatian Society of Medical Biochemists by processing of unknown samples 3 times *per annum*. The suitability of home-made control material for complete blood count was evaluated.

Materials and methods: The Croatian Institute for Transfusion Medicine prepared the home-made control material for complete blood count. A single portion of fresh blood is used given by voluntary donor having given consent to its intending use. A minor portion of the blood is tested for Hepatitis B and C as well as HIV and TP and was nonreactive for all these markers. After 30 minutes mixing in a way without making foam, 1.5 ml K_2EDTA molarity 1 mol/L is added and the mixing was continued next 2-5 minutes. The blood is distributed into suitable 7.5 x 12 mm sterile tubes, capped and stored at +2 °C to +8 °C. The samples were analysed for homogeneity and stability according to the ISO 13528 requirements.

Results: The home made-samples are delivered within 24 hours throughout Croatia in 200 medical biochemistry laboratories and are analyzed on 37 different types of analyzers from 11 different manufacturers. Assessment against consensus values and the percent allowed difference score is introduced for evaluation of obtained results. The high degree of inter-laboratory comparability is approved.

Conclusion: The home-made control material used in Croatian EQA scheme shows applicability to all haematological analyzers and provides a long term, retrospective assessment of laboratory performance and comparability of results on national level.

P09-08

Differential count in healthy newborns

Paradinović K (1), Milić M (1), Drmić S (2), Šerić V (1)

(1) University Medical Center Osijek, Department of Clinical Laboratory Diagnostics, Osijek, Croatia
(2) General Hospital of Vinkovci, Department for Biochemistry, Vinkovci, Croatia

Corresponding author: ksenija.paradinovic@gmail.com

Background: An accurate differential count is important in the diagnosis and monitoring of various clinical states. Our aim was to compare WBC differential morphology flags from haematology analyzer and differential blood count from microscopic slide examination in healthy newborns.

Materials and methods: CBC count was determined on haematology analyzer Sysmex XE2100. Differential was performed by examination of peripheral blood smear using light microscope. Slides were stained by May Grunwald Giemsa. We analysed 252 blood samples from healthy newborns aged 1-3 days.

Results: We compared flags for immature gran and left shift with nonsegmented neutrofiles, metamyelocytes, myelocytes. Average for nonsegmented neutrofiles was 6%. Compatible values (flags=pos, microscope=poz; flags=neg, microscope=neg) were in 105 cases. Noncompatibile values (flags=neg, microscope=pos) were in 11 cases and (flags=pos, microscope=neg) were in 140 cases.

Conclusions: Comparing the results, we noticed that the haematology analyser is more analytically sensitive. Microscopic slide examination should bee done in all samples flaged by haematology analayzer.

P09-09

The effect of the cellularity of the bone marrow aspirate sample for cytogenetic cultures

Tegg E (1), Raik E (2)

(1) University of Tasmania, Pathology, Hobart, Australia
(2) Royal North Shore Hospital, Haematology, Sydney, Australia

Corresponding author: Elizabeth.Tegg@utas.edu.au

Background: The role of cytogenetics in the diagnosis and prognosis of haematological malignancies is well recognised. The contribution made by the quality of the sample given to a cytogenetics laboratory to successfully provide diagnostic results is not.

Materials and methods: This was a retrospective control study made after a change to the bone marrow aspiration technique. The test group consisted of the first 65 patients who underwent a bone marrow biopsy requiring cytogenetic analysis after this change. The control group consisted of 65 patients who had undergone a bone marrow biopsy for cytogenetic analysis prior to this change. The parameters measured were disease type, morphological cellularity of the aspirated marrow, cell count of the cytogenetic sample, cytogenetic failure rate, number of metaphases obtained and the banding resolution achieved.

Results: Results were analysed using Microsoft Excel student t-test. The cellularity of the cytogenetic sample was significantly increased (1.23 x 10^9/L compared to 0.59 x 10^9/L; $P < 0.001$). The culture failure rate fell significantly from 15% to 2%. ($P < 0.01$). The optimal number of metaphases (20 metaphases per patient) was achieved in 75% of cases compared to 50% in the control group ($P < 0.04$). The banding resolution was significantly increased in the test group compared to the control group ($P < 0.04$).

Conclusions: Information in the literature is sparse when assessing the effect of BM cellularity on the ability to obtain a successful cytogenetic result. This study showed the positive effect of increasing the cellularity of the bone marrow aspirate sample for the successful cytogenetic analysis.

P09-10

Verification of the Body Fluid Application on the Sysmex XE-5000 Hematology Analyzer

Šiftar Z (1), Kardum-Paro M (1), Nazor A (1), Sokolić I (1), Kardum Skelin I (2), Flegar Meštrić Z (1)

(1) Merkur University Hospital, Institute of Clinical Chemistry and Laboratory Medicine, Zagreb, Croatia
(2) Merkur University Hospital, Institute of Clinical Cytology and Cytogenetics, Zagreb, Croatia

Corresponding author: zoran.siftar@gmail.com

Background: Aim of this study is to investigate the performance of BF application on the Sysmex XE-5000 hematology analyzer in differential diagnosis of exudates and transudates.

Materials and methods: The XE-5000 BF application provides white blood cell (BF-WBC) and total nucleated cell (BF-TC), as well as erythrocyte (BF-ERC) count. BF-WBC is further subcategorized in mononuclear and polymorphonuclear cells, BF-MN and BF-PMN. Performance of application was evaluated according to CLSI H56-A guidelines. Comparison to reference methods: microscopic cell counting in Neubauer chamber and differential on MGG stained smears after citospin preparation were done. Additionally, measurement of cells on XS2100 analyzer was included in comparative study. Automated cell counting was performed in Institute of Clinical Chemistry and Laboratory Medicine, whilst the microscopic in Institute of Clinical Cytology and Cytogenetics. It was done on various fluids (pleural effusion and ascites, dialysis fluid within peritoneal dialysis treatment) specimens. For correlation calculation Passing and Ba-block regression analysis is used.

Results: Repeatability of BF-WBC measurements was performed on 3 concentration levels. Coefficients of variation (CVs) were 21, 14 and 7.5, and met manufacturer specifications. BF-WBC linearity was proven for measurement range of 3 to 3420. Achieved coefficients of correlation with microscopic and measured counts on Sysmex XE 2100 for 35 specimens were 0.92 and 0.98, respectively. Obtained coefficient of 0.89 was found in compari-

son of automated and microscopic differential on 23 samples.

Conclusions: Evaluation of BF application on Sysmex XE-5000 showed significant improvement in the ability of automated hematology analyzers regarding body fluid cell count analysis.

P09-11

Integration of point-of-care hematology analyzers in the hospital and laboratory information system

Baršić I (1), Brkljačić V (1), Šrenger V (2), Majdenić K (3), Stojmilović S (3), Sertić J (1)

(1) University Hospital Center Zagreb, Clinical Institute of Laboratory Diagnostics, Zagreb, Croatia
(2) University Hospital Center Zagreb, Center for Informatics, Zagreb, Croatia
(3) IN2 Ltd, Health sector, Zagreb, Croatia

Corresponding author: ibarsic@kbc-zagreb.hr

Background: In 2000, our Institute started with implementing point-of-care testing at sites where it was important to decrease TAT, either for critical care patients or in order to increase efficiency and patients turnaround time. University Hospital Center Zagreb has 14 blood gas analyzers (GEM Premier 3000,Rapidlab 1265), 6 hematology analyzers (Sysmex pocH-100i), 7 coagulation monitoring system analyzers (CoaguChek XS, thromboelastometry ROTEM, aggregometry Multiplate®) and 105 glucometers (Accu Chek Inform II). Results from these devices are used to assess the current status of patients and often are not entered in the medical record, thus remaining unaccounted for in the final patient report with diagnostic and therapeutic procedures. Therefore, we initiated integration of point-of-care devices in the hospital and laboratory information system (HIS, LIS), starting with hematology analyzers.

Materials and methods: Sysmex pocH-100i is an automated hematology analyzer that provides 19-parameter complete blood counts with three-part leucocyte differential. It is connected via a local area network with LIS, further integrated into HIS. According to predefined criteria, LIS performes autovalidation and results are forwarded to HIS. In this way, results are available within a few seconds.

Conclusion: Integration of point-of-care devices in the hospital and laboratory information system allows the storage of patient results with electronic medical patient records and easier access to results when they are needed again. It also gives us the ability to have better control over reagent consumption and over results obtained from these devices.

P09-12

Blood loss from laboratory tests in adult hospitalized patients

Alatas O, Kocaturk E, Bekmez M, Bayrak C, Harmansa A

Eskisehir Osmangazi University, The Medical School, Medical Biochemistry, Eskisehir, Turkey

Corresponding author: oalatas@ogu.edu.tr

Introduction: Blood loss for laboratory tests can be important especially for inpatients. The aim of this study was to determine approximate blood loss of inpatients during their stay in hospital and whether this loss contributes to changes in hemoglobin levels.

Materials and methods: All patients hospitalized for eight or nine days in twenty five clinics of University Hospital of Eskisehir Osmangazi University in 2011 were reviewed retrospectively. We estimated blood loss by multiplying the number and approximate volumes of sampling tubes that used for laboratory analysis of 1792 adult inpatients. Moreover, the first and last sample hemoglobin levels of hospitalized patients were compared.

Results: The mean blood drawn from patients was estimated 35.27 mL. The highest blood volume was observed in patients from Chest Disease Clinic. Total tube number used for 1792 patient was 22,926, and the red top tube was consisted of 36%

of all tubes. Among the eight clinics which have more than fifty patients, Chest Disease Clinic was used the highest number of tubes. The first day median level of hemoglobin was reduced from 12.7 g/dL to 11.7 g/dL in eight days hospitalized patients and from 12.5 g/dL to 11.7 g/dL in nine days hospitalized patients.

Results: Blood loss from laboratory diagnostic testing is highly associated with changes in hemoglobin levels for inpatients and may contribute to anemia especially in high risk group patients. For this reason, unnecessary test requirements should be prevented and minimum test repetition time should take into consideration in order to reduce blood loss arise from laboratory diagnostic testing.

P10 – Haematology 2

P10-01

State of plasma and platelets hemostasis factors in patients with unstable angina

Markava I (1), Miadzvedzeva A (2), Helis L (1), Kaljadka M (3), Russkikh I (3)

(1) RPSC Cardiology, Laboratory of heart surgery, Minsk, Belarus
(2) RPSC Cardiology, 2nd cardiological department, Minsk, Belarus
(3) RPSC Cardiology, Clinical laboratory, Minsk, Belarus

Corresponding author: inga.gaponova@gmail.com

Methods and results: We studied 100 patients defined clinically as unstable angina (M/F = 60/40, 61 ± 12.2 years). Analyzed blood tests (platelet count, MPV- mean platelet volume, PDW – platelet distribution width), cardiac markers (CK-MB, myoglobin, troponin), biochemical analysis (C-reactive protein, BNP), coagulation blood tests, platelet aggregation test were taken at admission. Statistical analysis for the study was conducted using Statistica 6.0 (StatSoft. Inc,USA). The combined primary end point (all-cause mortality, nonfatal myocardial infarction, recurrent UA, urgent percutaneous coronary intervention or coronary artery bypass grafting) at 6 months occurred in 36 (36%). In these patients, the mean baseline MPV was greater than that in those without a primary outcome (9.1 ± 0.6 *vs*. 8.9 ± 0.7 fl, $P < 0.05$), PDW was less (13.4 ± 1.2 *vs*. 13.8 ± 1.1 %). In patients with adverse outcomes fibrinogen (6.3 ± 0.4 *vs*. 4.6 ± 0.5 g/L, $P = 0.02$), D-dimers (660 ± 24.1 *vs*. 382.2 ± 13.0 ng/mL, $P = 0.001$), BNP (103.5 ± 7.9 ng/mL *vs*. 61.6 ± 9.3, $P < 0.001$), von Willebrand factor (vWf) (186.2 ± 14.3 *vs*. 160.4 ± 20.1%, $P = 0.03$), spontaneous and induced (ADF, adrenalin) platelet aggregation ($P \leq 0.05$) were greater compare to patients without. The criteria for 6 months adverse events were MPV > 9.0 fL, fibrinogen > 6.4 g/L, D-dimer > 640 ng/mL, BNP > 110 ng/mL, vWf > 180%.

Conclusion: MPV can be used as a risk biomarker in prognosticating the 6 months outcomes for unstable angina. Patients adverse outcomes in unstable angina have increased level of fibrinogen, D-dimer, BNP, vWf, spontaneous and induced platelet aggregation

P10-02

Evaluation of platelet parameters for differential diagnosis of thrombocytosis

Peran N, Reškov Ž, Meniga D, Pamuković-Jaram D, Vesna S

General hospital Šibenik, Laboratory of Biochemistry and Hematology, Šibenik, Croatia

Corresponding author: nena.peran1@gmail.com

Background: There are two types of thrombocytosis: primary and secondary. Primary thrombocytosis (PT) is caused by a chronic myeloproliferative disorders (CMPD) and secondary thrombocytosis is called reactive thrombocytosis (RT) since it is associated with inflammatory states. Differential diagnosis of thrombocytosis is not always obvious. In automated blood cell analyzers various platelet parameters can now be measured. In this study, we analyzed whether 6 platelet parameters can be used for the differentitation of PT from RT.

Materials and metods: The platelet counts, mean platelet volume (MPV), plateletcrit (PCT), platelet distribution width (PDW), mean platelet mass

(MPM), mean platelet component concentration (MPC) and large platelets (LPLT) were studied in 40 patients with RT, 18 patients with PT and 60 normal control. Platelet parameters were measured by ADVIA 2120 (Bayer Diagnostics, USA). Significance was determined using the Mann-Whitney test.

Results: Patients with CMPD had significantly higher MPV, PDW, L-PLT, PCT, MPM than those with reactive thromboytosis. There was no significance difference in MPC between patients with PT and RT, but in both groups MPC was significantly lower than in control group. Also, there was no difference in MPM between patients with primary thrombocytosis and control group.

Conclusion: The platelet parameters are useful for the differential diagnosis of thrombocytosis. High MPV, PDW, and L-PLT with high platelet counts suggest primary thrombocytosis.

P10-03

Evaluation of in-house normal pool plasma for partial thromboplastin time mixing study on an ACL TOP

Costa P, Reis J, Silva C, Simão L, Diamantino I, Valido F

IPOC FG EPE, Clinical Pathology, Coimbra, Portugal

Corresponding author: jacreis@iol.pt

Background: Normal pool plasma plays a critical role on the safe outcome on testing prolonged partial thromboplastin time (PTT). Mixing study for qualitative inhibitor detection is commonly used on routine laboratory testing, allowing detection or exclusion of an inhibitor as differentiation from primary factor deficiency.

Materials and methods: In-house normal pool plasma (In-house) was obtained from 50 healthy donors immediately after collection and centrifugation (3000 rpm; 10 minutes), properly mixed and stored at - 60 °C. Commercial normal pool plasma from ILTM Normal Control® (IL) and StagoTM Pool Norm® (Stago) were used for result comparison. 78 samples with prolonged PTT (ratio > 1.20) were properly mixed in equal volume with the normal pool plasmas and evaluated for PTT. Correlation was evaluated by pearson's correlation factor. Mean and standard deviation (SD) were analyzed for all groups. T-Test was performed for group comparison.

Results: Mean ± SD for the mixing tests of the three groups as ratio: In-house – 1.16 ± 0.13, IL – 1.23 ± 0.17, Stago – 1.14 ± 0.16. T-Test analysis between groups: In-house *vs*. IL – 0.0052, In-house *vs*. Stago – 0.4765, IL *vs*. Stago – 0.0016. All groups showed a good pearson correlation: In-house *vs*. IL – 0.7683, In-house *vs*. Stago – 0.8883, IL *vs*. Stago – 0.8349.

Conclusions: A critical evaluation of T-Test and correlation between in-house normal pool indicates great similarity of results compared to those obtained with the commercial plasma pools tested, thus suggesting that a careful selection of donors allows preparing in-house normal pool plasma that fits the quality demands of routine laboratory mixing PTT tests.

P10-04

Comparison of two methods for measuring FVIII levels in hemophilia A patients

Miloš M (1), Coen Herak D (1), Zupančić-Šalek S (2), Zadro R (1)

(1) University hospital centre Zagreb, Department of laboratory diagnostics, Zagreb, Croatia
(2) University hospital centre Zagreb, Department of medicine, Zagreb, Croatia

Corresponding author: marijamilos1@yahoo.com

Background: Correct determination of FVIII activity is essential for the assessment of severity of hemophilia A, as well as for patient-tailored treatment strategy. The most commonly performed assay for measuring FVIII activity is one-stage clotting assay. Several groups of authors have reported discrepancies between one-stage and chromogenic assay for FVIII determination.

Materials and methods: We have compared the one-stage and chromogenic FVIII assay (Siemens, Marburg, Germany) in 100 hemophilia A patients (56 severe and 44 non-severe) and 101 healthy male subjects.

Results: Good correlation between assays was obtained in healthy subjects as well as in severe and non-severe group of patients (coefficients of correlation 0.607; 0.904 and 0.875, respectively), but statisticaly significant difference was found in all groups. According to Bland & Altman the mean differencies between assays in healthy subjects, severe and non-severe patients were 17.6%, 47.8% and 61.7%, respectively. Usually, the results with chromogenic assay were lower than results with one-stage clotting assay. Similar correlation with clinical parameters (age at first joint bleed, number of joints with hemophilic arthropathy, number of annual joint bleeds and annual FVIII consumption) was found: coefficients of correlation between 0.568 and 0.688 for one-stage and between 0.521 and 0.619 for chromogenic assay.

Conclusions: Two assays mostly show good correlation but clinically important discrepancies could be observed. It is recommended that all hemophilia centres have available both types of assay. Chromogenic assay should be used in the cases of normal aPTT and one-stage FVIII activity with presence of personal or familiy history of bleeding.

P10-05

Design and application of lupus anticoagulant diagnostic algorithm

Banys V, Cimoš R

Vilnius University Faculty of Medicine, Department of Physiology, Biochemistry, Microbiology and Laboratory Medicine, Vilnius, Lithuania

Corresponding author: valdas.banys@santa.lt

Background: Lupus anticoagulant (LA) diagnostics is highly complicated. Laboratory's responsibility is to select appropriate assays as recommended by certain published guidelines. Results must be interpreted properly, which is not an effortless and easy task. Aim of study was to design LA diagnostics algorithm, implement it in routine work of local laboratory, evaluate difference of diagnostic capabilities between single assays (aPTT, dRVVT) and complex of methods (assays plus indexes) used in new LA algorithm.

Materials and methods: In total 68 persons were selected, 40 relatively healthy for calculation of dRVVT reference values, and 28 suspected to have positive LA. aPTT, dRVVT screen and confirm, mixing studies of aPTT and dRVVT screen were performed. dRVVT normalization ratio, Rosner index (RI) of aPTT, dRVVT screen, correction index (CI) of dRVVT were calculated.

Results: Plasmas with suspected LA gave different numbers of positive results when different interpretative breakpoints were used: aPTT RI >15% was determined in 10.7%, dRVVT screen RI > 15-17.9% and dRVVT CI > 10-75% of patients.

Conclusions: Narrower than manufacturer's dRVVT reference intervals resulted in more positive LA results, most probably meaning greater number of false positives. No statistically significant difference in sex dependant dRVVT reference values was found. LA algorithm gave much less positive LA results than merely results of single assays. Despite using different reference values LA algorithm gave the same number of positive LA results and positives were detected in the same specimen.

P10-06

Relationship between homocysteine level and MTHFR haplotype in patients with thromophilia

Beletic A (1), Djordjevic V (2), Canic I (1), Mirkovic D (3), Radojkovic D (2), Majkic-Singh N (3)

(1) Clinical Center of Serbia, Belgrade, Serbia, Center for Medical Biochemistry, Belgrade, Serbia
(2) Institute of Molecular Genetics and Genetic Engineering, University of Belgrade, Serbia, Laboratory for Molecular Biology, Belgrade, Serbia
(3) Clinical Center of Serbia, Belgrade and Faculty of Pharmacy, University of Belgrade, Serbia, Center for Medical Biochemistry, Belgrade, Serbia

Corresponding author: abeletic@yahoo.com

Background: Increased homocysteine (Hcy) concentration is associated with increased risk for venous and arterial thrombosis. Reduced methylentetrahidrofolate reductase (MTHFR) activity, due to the presence of C677T and A1298C polymorphisms, is one of the causes of hyperhomocysteinema. In a group of patients with thrombophilia, Hcy leveles were compared between MTHFR haplotypes.

Materials and methods: Study enrolled 30 patients (12 men, 18 women). Hcy was measured using HPLC technique with fluorescent detection. PCR amplification and reverse allele-specific oligonucleotide hybridization were employed for MTHFR haplotype determination. Statistical analyses included Kruskal-Wallis and Mann Whitney U tests.

Results: The following distribution of MTHFR haplotypes was observed: 677CC/1298AA was present in 4 (median Hcy = 10.8 μM), 677CC/1298AC in 7 (median Hcy = 11.7 μM), 677CC/1298CC in 3 (median Hcy = 9.7 μM), 677CT/1298AA in 4 (median Hcy = 10.4 μM), 677CT/1298AC in 8 (median Hcy = 11.2 μM) and 677TT/1298AA in 4 patients (median Hcy = 14.6 μM). No statistically significant difference was observed between Hcy concentration corresponding to the detected haplotypes.

Conclusions: In a group of patients with thrombophilia, no significant difference in Hcy concentration, related to MTHFR haplotype, was detected. This finding further implies that the Hcy measurement has greater clinical importance than MTHFR genotyping, but such assumption has to be tested in larger studies.

P10-07

Validation of Hemoclot® Thrombin Inhibitors assay for the new oral anticoagulant dabigatran

Lilja J, Leinonen J, Saarela E, Lassila R, Joutsi-Korhonen L

HUSLAB Laboratory Services, Helsinki University Central Hospital, Coagulation Disorders Unit, Clinical Chemistry and Hematology, Helsinki, Finland

Corresponding author: jari.lilja@hus.fi

Background: The direct thrombin inhibitor dabigatran does not require laboratory monitoring routinely. However, under special circumstances (e.g. renal insufficiency, bleeding complication, thrombosis, major surgery) testing may be crucial. Routine coagulation assays prothrombin time (PT), INR and activated partial thromboplastin time (APTT) are of limited value. We validated a commercial assay based on diluted thrombin time (TT).

Materials and methods: Hemoclot® Thrombin Inhibitors test with control samples (Hyphen, Aniara) was evaluated for repeatability and linearity. Patient results (N = 30) were compared with Owren PT (Nycotest PT, Medinor), APTT (Actin FSL, Siemens Healthcare Diagnostics) and TT (Thrombin Reagent, Siemens). Coagulation analyser BCS XP (Siemens) was used.

Results: Intra-assay repeatability (CV%) tested with patient samples was 7.2%. Inter-assay repeatabilities with control samples were 10.1% (at 130 μg/L) and 7.9% (at 290 μg/L). The method was linear within the concentration range 50-470 μg/L (R = 0.997). Correlation between Hemoclot® and APTT was moderate (R^2 = 0.77), however there was considerable variation in APTT results, likely reflecting the clinical diversity of the patients. Even

at the highest concentration (490 µg/L), APTT was prolonged only to 62 s (reference values 23-33 s). Correlation with PT was poor ($R^2 = 0.23$) expectedly. TT exceeded the measurement range (> 140 s) already at low concentrations (< 50 µg/L).

Conclusions: Hemoclot® Thrombin Inhibitors demonstrated acceptable repeatability and linearity and seems suitable for dabigatran assessment. Here, APTT poorly estimated dabigatran concentration. Interpretation of the results depends on the clinical situation and timing. Yet, the cut-off values or safety limits in different clinical conditions remain unestablished.

P10-08

Indicators of activation of the coagulation system in children with Henoch-Schönlein purpura

Obuljen J (1), Kapović A (2), Linarić I (1), Margetić S (3), Magdić B (1), Žižić V (1)

(1) Children's Hospital Zagreb, Department of Laboratory Diagnostics, Zagreb, Croatia
(2) Children's Hospital Zagreb, Department of Pulmology, Alergology and Rheumatology, Zagreb, Croatia
(3) Children's Hospital Zagreb, Department of Laboratory Diagnostics, Zagreb, Croatia

Corresponding author: jasna.obuljen@xnet.hr

Background: Henoch-Schönlein purpura (HSP) is the most common vasculitis of childhood, affecting skin, joints, gastrointestinal tract and kidneys with clinical manifestations of bleeding tendency. The aim of this study was to evaluate global coagulation tests and D-dimer concentration as laboratory signs of activated coagulation system at first presentation of HSP.

Materials and methods: The study included 21 paediatric patients with HSP, median age 6 (range 3-16). Global coagulation tests PT, APTT, TT, fibrinogen were determined with coagulometric methods (Sysmex CA-560 analyzer, Siemens, Germany). D-dimer concentration was measured with microparticle enzyme immunoassay (Axsis Shield Diagnostics Ltd., USA) on Axsym analyzer (Abbott, USA).

Results: The results of coagulation tests expressed as median (95% confidence interval (95% CI), interquartile range (IQR)) were: PV ratio 0.96 (0.87-1.02, 0.85-1.04); APTV (sec) 26.7 (24.6-28.3, 24.0-28.5); fibrinogen (g/L) 3.7 (3.2-4.6, 3.1-4.8), TT (sec) 16.5 (16.1-17.6, 15.9-17.9), D-dimer (mg/L) 5.3 (1.8-7.7, 1.6-8.1). This study showed no alterations of PT, APTT and TT tests in HSP patients, fibrinogen was increased in 6/21 patients while D-dimer levels were increased in all 21 patients. In patients grouped according to the number of organs involved (2, 3 and 4) D-dimer concentrations were as follows: 2 organs involved: 2.9 mg/L (IQR = 1.4-7.5); 3 organs involved: 7.0 (IQR = 2.9-9.0) and 4 organs involved: 6.5 (IQR = 3.9-9.0), but there were no significant differences in D-dimer concentrations between groups.

Conclusion: Increased D-dimer concentrations in all 21 paediatric patients with HSP suggest that activation of coagulation including hyperfibrinolysis secondary to the endothelial damage to be a typical feature of HSP.

P10-09

Pre-analytical routines in coagulation testing: are guidelines followed?

Kristoffersen A (1), Stavelin A (2), Vannes S (1), Berge Kristensen GB (3)

(1) Laboratory of Clinical Biochemistry, Haukeland University Hospital, Bergen, Norway
(2) Norwegian Quality Improvement of Primary Care Laboratories (NOKLUS), Department of Public Health and Primary Health Care, University of Bergen, Bergen, Norway
(3) Norwegian Clinical Chemistry EQA Programme, Norwegian Clinical Chemistry EQA Programme, Bergen, Norway

Corresponding author: ann.kristoffersen@helse-bergen.no

Background: It has been documented that about 70% of errors in laboratory medicine occur in the preanalytical phase. The aim of this survey was to study preanalytical conditions of routine hemostasis testing in Norwegian laboratories, and compare them with current guidelines.

Materials and methods: Hospital laboratories in Norway (N = 69) were invited to fill in a web-based

questionnaire regarding preanalytical routines for routine hemostasis testing. The first part focused on instruments and reagents for the different coagulation analyses used, and the second part focused on routines regarding venipuncture (needle gauge, citrate concentration, use of stasis, filling volume), handling of the sample before analysis, as well as routines regarding detection and handling of sample clot, high/low hematocrit, hemolysis, bilirubinemia or lipemia. The third part focused on storage and stability and the handling of samples from primary care or other hospital laboratories.

Results: 57 of 69 laboratories responded. There was good agreement regarding needle gauge, temperature in the centrifuge and type of glass used for sample collection

(3.2% Na-citrate), all more or less following the CLSI guidelines (H21-A5). However, large differences in practice were seen amongst the participants regarding centrifugation speed and duration, accepted fill volumes of the collection tubes accepted and accepted sample material (fresh or frozen plasma or citrated blood) and stability of the blood samples. In addition, there were few routines for detection of clot, pathological hematocrit, hemolysis, bilirubinemia and lipemia.

Conclusions: Wide variation is seen in preanalytical routines in hemostasis testing, often not according to the CLSI guideline.

P10-10

Characterization of blood donors with high haemoglobin concentration

Magnussen K (1), Hasselbalch H (2), Ullum H (3), Weis Bjerrum O (2)

(1) Copenhagen University Hospital, Rigshospitalet / Hvidovre Hospital, Clinical Immunology / Blood Centre, Copenhagen, Denmark
(2) Copenhagen University Hospital, Roskilde Hospital, Haematology, Copenhagen, Denmark
(3) Copenhagen University Hospital, Rigshospitalet, Clinical Immunology / Blood Centre, Copenhagen, Denmark

Corresponding author: karin.magnussen@regionh.dk

Background: The prevalence and causes of high haemoglobin concentration among blood donors are poorly described. This study aimed to characterize and develop an algorithm to manage the donors with polycythaemia.

Matrials and methods: Between November 2009 and November 2011 blood donors with repeated B-Hb above the WHO limit for polycythaemia vera (10.2 and 11.5 mmol/L/ 16.5 and 18.5 g/dL for women and men, respectively) were offered consultations by a hospital-based haematologist. At consultation haematocrit, MCV, P-erythropoietin, P-ferritin, B-Hb, platelet- and leukocyte count, JAK2 V617 and JAK2 exon12 analysis were performed, in addition to other routine parameters.

Results: Among 46 donors with repeated high B-Hb, 39 had a history of smoking, which may contribute to polyglobulia. Two had PV, 5 had severe hypertension, one of them because of kidney artery stenosis, and two had diabetes mellitus. Ten donors were deferred and of the 36 donors that were not deferred, 30 donated again before May 2012, where the B-Hb was significantly lower.

Conclusion: Thus, we found a high morbidity among these donors, and recommend JAK2 V617 and JAK2 exon12 screening and clinical investigations for donors with concurrent high B-Hb, high haematocrit and iron deficiency. Also we recommend these donors to reduce smoking to reduce the risk of thrombosis in general.

P10-11

Blood donors with thalassemia trait in a blood bank outside the thalassemia belt

Magnussen K

Copenhagen University Hospital, Rigshospitalet / Hvidovre Hospital, Clinical Immunology / Blood Centre, Copenhagen, Denmark

Corresponding author: karin.magnussen@regionh.dk

Background: By far the most frequent reason for Danish blood donors to have a haemoglobin level below the level of acceptance for blood donation is iron deficiency. Hence in all low haemoglobin-

donors ferritin is measured. If haemoglobin remains low by the next visit or if the low haemoglobin cannot be explained by low ferritin, further tests are done. Five donors with mean cell volume (MCV) ≤ 78 fL, not explained by iron deficiency are described.

Materials and methods: Samples from donors with unexplained microcytaemia, were sent to Centre for Haemoglobinopathies, where Hb-electrophoresis and nucleic acid investigations were done.

Summary: With increasing globalisation donors heterozygous for thalassemia may turn up even in Nordic blood banks. Their haemoglobin level will be in the low normal area, often below the lower limit for donation, except for the α3,7 deletion where the haemoglobin level often is normal. Heterozygous thalassemia is rarely of clinical consequence, except in pregnancy where genetic counselling may be warranted. Provided that the haemoglobin level is above the limit for acceptance, people with thalassemia trait may become blood donors.

P10-12

Temporary impact of blood donation on physical performance in moderately physically active men

Magnussen K (1), Ziegler A (2), Grand J (2), Helge J (2)

(1) Copenhagen University Hospital, Rigshospitalet / Hvidovre Hospital, Clinical Immunology / Blood Centre, Copenhagen, Denmark
(2) University of Copenhagen, Faculty of Health Sciences, Copenhagen, Denmark

Corresponding author: karin.magnussen@regionh.dk

Background: Donation of blood negatively affects maximal oxygen consumption (VO2max) and endurance, and donors ask about the duration and degree of reduction.

Materials and methods: Nineteen non-anaemic, physically active male blood donors age 33(24–43) were included. To determine VO2max, subjects performed a standard incremental bicycle ergometer VO2Max test. Endurance was tested using a self-paced 3 km treadmill run. Subjects were tested 2 days before and 3, 7, 14, and 28 days after donation and were drawn to analyse haematological - and iron-parameters.

Results: Haemoglobin was reduced by 9.3% at day 3. After this Hb increased, and by day 28, three of 15 donors had reached pre-donation Hb level. Ferritin declined 51% from 94.8 ± 18.4 µg/L before donation to 46.8 ± 10.4 µg/L at day 14 and remained below baseline throughout the study. VO2max declined by 6.5% from 49.7 ± 2.1 mL O_2 / kg/min to 45.6 ± 2.1 mL O_2 /kg/min at day 3 compared with before donation. Subsequently VO2max gradually increased to 49.3 ± 2.2 mL O_2 / kg/min, at day 14, thereby returning to baseline. The 3 km time trial performance declined by 5.6% from 13:55 ± 00:46 minutes before donation to 14:42 ± 01:12 minutes at day 3. At day 14 time trial performance was 13:52 ± 00:59 minutes, the same as before donation.

Summary: Haemoglobin concentration was reduced by 9.3% and ferritin by 51%. Endurance was reduced by 5.6% and VO2max by 6.1% and reached pre-donation level by day 14, It seems from our study that plasma volume expansion may partly compensate for the loss in haemoglobin.

P11 – Immunology

P11-01

Comparative analysis of turbidimetry and nephelometry methods for the measurement of immunoglobulin

Cachapuz I (1), Moreira S (2), Neves E (3), Lima I (3), Cerveira C (3), Alves V (1)

(1) Matosinhos Local Health Unit, Clinical Pathology Service, Oporto, Portugal
(2) ESTSP, Oporto, Portugal
(3) Centro Hospitalar do Porto, Clinical Immunology Service, Oporto, Portugal

Corresponding author: cachapuz.isabel@gmail.com

Background: The analysis of the protein content in urine samples is often required in the clinical practice. Nephelometry and turbidimetry are alternative methods, frequently used in the laboratory routine, and its choice depends on various factors including the cost, availability and characteristics of the laboratory equipments. A discussion is still ongoing, about the most suitable method, considering its sensibility and specificity, for the measurement of proteins in very low concentration levels, like monoclonal components in urine samples. The aim of this study is to compare the results of Immunoglobulin G (IGG), kappa (CLK) and lambda (CLLl) light chains concentration in urine samples, obtained by turbidimetry and nephelometry.

Materials and methods: Experimental and retrospective study of 24-hour urine random samples, from 51 patients of Pedro Hispano Hospital, collected for monoclonal protein excretion screening. IGG, CLK and CLL analysis were performed by turbidimetry (Abbott Diagnostics, Architect C 8000®) and by nephelometry (Beckman Coulter IMMAGE® equipment for IGG and Siemens Dade Behring BN II equipment for CLK and CLL). Statistic analysis was performed with SPSS (vs 18) software.

Results: Correlation values between results obtained by turbidimetric and nephelometric methods were 93,5%, 99,0%, 99,0% respectively for CLK, CLL and lGG, with p values 0.065 (CLK), 0.570 (CLL), 0.004 (IGG). Significance level was 0.05.

Conclusions: Our results suggest that the performance of both methods is similar for the three parameters under study. Therefore, turbidimetry, the method most commonly used in automated large scale laboratory routine models, like COREIabs, is suitable for this propose.

P11-02

Comparison of rheumatoid factor analysis by nephelometry (BN II) and turbidimetry (ADVIA 2400)

Garcia Moreira V, Fernandez Leivas A, Bachiller Sister M, Llorente Torres A, Fernandez Rodriguez E

Hospital de Cabueñes, Servicio de Analisis Clinicos, Gijon, Spain

Corresponding author: eloyfr@gmail.com

Background: Detection of rheumatoid factor (RF) is a useful method in the differential diagnosis of rheumatic diseases, primarily used to help diagnose rheumatoid arthritis or Sjögren syndrome. Changes in serum RF levels may be used as indicators of disease activity, but they are also proving useful in monitoring responses to therapy.

Materials and methods: Levels of FR were analyzed in 75 serum samples in parallel by our usual immunonephelometric method in a BNII analyzer (Siemens) and a new immunoturbidimetric method in Advia2400 (Siemens). Data were statistically analyzed using the MedCalc statistical package by agreement kappa statistic, Spearman correlation and regression of Passing-Bablok. To categorize the results were considered negative values RF < 14 IU/mL in Advia2400 method and < 15.9 IU/mL in the nephelometric assay, and other results were considered positive.

Results: The observed agreement was 91% (expected by chance: 55%), yielding a kappa index of 0.795 (95%CI: 0.624-0.966). We calculated Spearman correlation for positive samples (N = 35) be-

tween the ratios (value/upper reference limit) of both methods: 0.810 (0.573-0.922) (P < 0.01). The Passing-Bablok regression equation was: Advia2400 = 3.56 + 1.09 x BnII (95% CI slope: 0.794-1.55; 95% CI intercept:-9.26-9.47), so no bias was evident.

Conclusion: According to the results, the strength of categorical agreement between both methods can be considered "strong" (Ladis-Koch criterion). That is, the results are qualitatively comparable. However, the results are not quantitatively interchangeable. The turbidimetric RF Advia2400 assay is a reasonable alternative to the nephelometric, as it shows a good correlation, besides being cheaper and faster.

P11-03

Evaluation of C3 and C4 immunoturbidimetric assays on ABBOTT Architect ci16200 analyzer

Knezevic B, Mladina B, Čepić K, Surjan L

University Hospital Split, Department of Laboratory Diagnostic, Split, Croatia

Corresponding author: bknezevic@net.hr

Background: Our laboratory wished to consolidate C3 and C4 testing by converting from immunonephelometry to immunoturbidimetry. We evaluated analytical performance of Abbotts C3 and C4 immnoturbidimetric assays on Abbott Architect ci16200 chemistry/immunochemistry analyzer.

Materials and methods: Analytical evaluation included determination of within - run (N = 20), between - run (N = 20) imprecision, inaccuracy (N = 20) using control materials and method comparison on human samples (N = 90) comparing Architect ci16200 and Siemens BNProSpec immunonephelometer. For the sample comparison Passing-Bablok and Bland-Altman analyses were performed. For the evaluation of clinical significance, reference-change values (RCV) at two control levels were calculated.

Results and conclusions: Results obtained for within run imprecision: C3 (L1 0.8%, L2 0.8%), C4 (L1 0.8%, L2 0.8%), between run imprecision: C3 (L1 1.9%, L2 1.2%), C4 (L1 2.1%, L2 3.9%) and inaccuracy: C3 (L1 3.7%, L2 4.08%), C4 (L1 2.2%, L2 6.6 %), satisfied desirable specifications for I%, B% and TE% derived from biological variation. Regression equations with 95% CI obtained from Passing-Bablok analysis were as followed: C3 y = - 0.21 (-0.24-(-0.17)) + 1.7 (1.13 – 1.20)x, C4 y = 0.00 + 1.10 (1.13-1.20)x. Bland-Altman plot showed C3 values on average 2.2% lower (limits of agreement -9.7-5.2%) and C4 on average 9.6% higher (limits of agreement 5.1-14.1%) on Architect ci16200. Although Passing-Bablok regression showed statistically significant difference between methods, Bland-Altman plot showed differences clinically insignificant if compared to RCV values.

P11-04

Comparison of serum total IgE tests performed by four different assay systems

Yoon S (1), Kim S (2)

(1) U2Bio, Department of Laboratory Medicine, Seoul, Korea, (South) Republic of
(2) Yonsei University College of Medicine, Department of Laboratory Medicine, Seoul, Korea, (South) Republic of

Corresponding author: sykim@yuhs.ac

Background: In vitro testing is commonly used to diagnose allergies and predict allergic tendency especially in pediatrics. Intermethod comparisons between four different commercial tests for serum total IgE had only been evaluated by limited studies. To determine whether IgE levels derived from different assays are precise and equivalent to those measured by ImmunoCAP.

Materials and methods: Precision was determined following Clinical and Laboratory Standards Institute EP5-A2 using patients' pooled se-

rum. Two levels materials were analyzed in duplicates at two separate times per day for 20 days in each four systems. We performed interassay comparisons using 132 Korean patients who visited one tertiary care hospital in Seoul. For each deidentified sample, total IgE levels were measured using four different assay systems(bioMérieux VIDAS, Siemens Immulite2000, Roche Cobas e 601, Phadia ImmunoCAP). Results were analyzed to determine whether IgE measurements were comparable.

Results: Four clinically used total serum IgE assays showed excellent precision performance, with coefficients of variation (CVs) below 9%. After 132 paired comparison test, Immulite2000 ($y = 1.02x - 1.0$, $R^2 = 0.9806$) and Cobas e 601 ($y = 1.14x+-0.94$, $R^2 = 0.9913$) showed good correlation with ImmunoCAP assay. Although some minor outliers were noted. VIDAS ($y = -4.92x + 0.91$, $R^2 = 0.9423$) showed significant deviation from linearity with ImmunoCAP by Passing – Bablok analysis.

Conclusions: The Immulite2000 and Cobas e601 total IgE were showed reliable performance and comparable result with ImmunoCAP assay. VIDAS showed underestimated trend in total IgE values. We should take into account the intermethod differences between those assays for clinical applications.

P11-05

Imunonephelometric quantification of monoclonal protein

Šegulja D (1), Matišić D (1), Batinić J (2), Nemet D (2)

(1) University Hospital Centre Zagreb, Department of Laboratory Diagnostics, Zagreb, Croatia
(2) University Hospital Centre Zagreb, Department of Hematology, Zagreb, Croatia

Corresponding author: dragana.segulja@gmail.com

Introduction: Hevylite is a new test for imunonephelometric quantification of monoclonal protein. The reagent consists of polyclonal antibodies produced in sheep. Since the target of this antibodies are unique junctional epitopes between the heavy chain and light chain constant regions, antibodies can separately identify the different light chain types of each Ig class (IgG kappa, IgG lambda). Aim of the study was evaluation of new test for monoclonal protein (M protein) quantification.

Materials and methods: Study included 20 samples with already detected M protein. In all samples were measured total imunoglobulin A, G and M (Cobas 6000, Roche) and done capillary zone electophoresis (Capillarys2, Sebia) and immunofixation electrophoresis (Hydrasys, Sebia). After detection, M protein is quantified with The Binding Site test Hevylite on nephelometer Siemens BNII.

Results and conclusions: Monoclonal protein were detected even when immunoglobulins were in reference ranges. When detected M protein is IgA class, densitometric determinated gamma globulins can not be usefull in quantification of M protein. Numerical results of total immunoglobulin concentrations and M protein are not comparable. Ratio kappa/lambda may be a good indicator of clonality. Unquestionable is importance of capillary zone electroforesis in identifying monoclonal gammapathies as simple and inexpensive routine technique in clinical laboratory. But, sometimes when the M protein (expecially IgA) migrates in the beta reagion, with either transfferin or C3, only quantification of the M protein will provide adequate follow-up. Considering the price of reagent the test probably will not find use in screening for monoclonal protein but will be usefull in monitoring the disease course.

P11-07

YKL-40 correlates with pro-inflammatory cytokines in rheumatoid arthritis

Kazakova M

Medical University - Plovdiv, Medical Biology, Plovdiv, Bulgaria

Corresponding author: kazakova25@abv.bg

Background: The aetiology of rheumatoid arthritis remains unknown, although it is estimated that autoimmune mechanisms play a major role in the pathogenesis of the disease. YKL-40 is a candidate for a novel inflammatory marker. It is secreted by activated macrophages and neutrophils, synovial cells, arthritic chondrocytes and cancer cells. In this study we evaluated YKL-40 levels in serum and synovial fluid of patients with rheumatoid arthritis in comparison with the concentration of pro-inflammatory cytokines.

Materials and methods: We examined serum and synovial YKL-40, IL-1β and TNF-α levels in 37 rheumatoid arthritis patients, aged 53.14 ± 2.73. The concentrations of these markers were measured by ELISA.

Results and conclusions: The levels of YKL-40, IL-1β and TNF-α in patients were remarkably higher compared to the healthy group ($P < 0.01$). A significant correlation between serum and synovial YKL-40 levels and concentrations of IL-1β and TNF-α in patients with rheumatoid arthritis was observed ($P < 0.01$). These pro-inflammatory cytokines are involved in the pathogenesis of rheumatoid arthritis and are targets for the therapeutic treatment. It is shown that IL-1β and TNF-α could induce secretion of YKL-40 by chondrocytes. We determined a strong correlation between serum YKL-40 concentration and the conventional biochemical marker for assessment of disease activity - C-reactive protein (CRP) ($P = 0.004$; $r = 0.582$). Our data suggest potential involvement of YKL-40 in inflammation and disease activity of rheumatoid arthritis. Acknowledgments The study is supported by grants NO–1/2009 and NO–1/2010 from Medical University– Plovdiv.

P11-08

Epstein-Barr virus infection resembling autoimmune liver disease in 17-year-old girl – case report

Tesija Kuna A (1), Zaja-Franulovic O (2), Vukasovic I (1), Lesar T (2), Vrkic N (1)

(1) Medical School University Hospital Sestre Milosrdnice, Clinical Institute of Chemistry, Zagreb, Croatia
(2) Medical School University Hospital Sestre Milosrdnice, Department of Pediatric Gastroenterology and Hepatology, Zagreb, Croatia

Corresponding author: andrea.kuna@gmail.com

We report a case of a 17-year-old girl admitted to Department of Pediatric Gastroenterology and Hepatology for chronic intermittent diarrhea with significant weight loss. Biochemical liver lesion confirmed with imaging techniques was found characterized with cholangitic pattern. High value of fecal calprotectin and hypoalbuminemia suggested inflammatory bowel disease with liver lesion as an extraintestinal manifestation. Autoantibody profile corresponding to autoimmune hepatitis type I was found including positive antinuclear (ANA) and smooth muscle (SMA) antibodies. Antimitochondrial antibodies (AMA) were negative on immunofluorescence test but high reactivity was observed using line immunoassay with both native AMA-M2 antigen and recombinant fusion protein AMA-M2-3E. Infective serology on admission revealed recent primary Epstein-Barr virus (EBV) infection with viral capside antigen (VCA) IgM and IgG positive, EBV early antigen (EA) IgG negative and EBV nuclear antigen (EBNA) IgG positive. Primary suspicion of ulcerative colitis was rejected regarding the normal endoscopic and histopathological findings of ileocolonic mucosa and spontaneous resolution of clinical symptoms, biochemical and ultrasonographic abnormalities. Repeat testing after 3 months revealed persistently positive ANA and SMA but absence of reactivity with AMA antigens on line immunoassay. Serologic tests documented seroconversion further supporting the diagnosis of EBV primoinfection. Repeated PCR analysis was negative for EBV DNA.

EBV infection as trigger for autoimmune disease has been attributed to occurrence of cross-reactive antibodies, due to the mimicry of epitopes between host and EBV proteins. We presented an example of EBV primoinfection resembling autoimmune liver disease although follow up is suggested due to the persistently positive ANA and SMA.

P11-09

Concentration of IgE antibody in nasal lavage in allergic rhinitis and non-allergic rhinitis

Bokulić A (1), Bukovec Megla Ž (1), Kalogjera L (2), Tomljenović D (2), Vagić D (2), Zurak K (2)

(1) University Hospital Centre "Sestre milosrdnice", Laboratory of Endocrinology, Department of Oncology and Nuclear Medicine, Zagreb, Croatia
(2) University Hospital Centre "Sestre milosrdnice", Department of Otorhinolaryngology and Head and Neck Surgery, Zagreb, Croatia

Corresponding author: zeljka.bukovec@kbcsm.hr

Background: Molecular and cellular inflammation mechanisms in nasal mucosa in allergic rhinitis (AR) and non-allergic rhinitis (NAR) have not yet been clarified. To contribute to their understanding and developing of differential diagnostics, concentration of total IgE was measured in nasal lavage in subjects with allergic rhinitis and non-allergic rhinitis. The objective of this study was to measure concentration of IgE antibody in nasal lavage and determine possible correlations.

Materials and methods: Study included 60 patients of both sexes aged 18-65. Patients were divided in two groups, according to diagnosis of allergic or non-allergic rhinitis (N = 30). Nasal lavage samples were taken by modified Nacleir method. Nasal cavity was flushed with 3 mL of saline solution (0.9% NaCl). The concentration of total IgE was measured by fluoro enzyme immunoassay (FEIA) on UniCAP 100 (Phadia AB, Uppsala, Sweden). The results were evaluated by non-parametric Mann-Whitney U-test. The values $P < 0.05$ were considered statistically significant.

Results: Results were expressed as median and interquartile range (Q1 and Q3). The concentration of total IgE was higher in patients with allergic rhinitis than in patients with non-allergic rhinitis: 40.60 kU/L (23.78-54.43) *vs*. 2.55 kU/L (2.05-5.80), respectively; $P < 0.001$.

Conclusions: The results showed significantly higher concentration of IgE antibody in nasal mucosa in patients with allergic rhinitis than in patients with non-allergic rhinitis. Local IgE-mediated inflammation plays important role in allergic rhinitis and measuring the concentration of local IgE antibodies in nasal lavage may contribute to diagnostics of allergic disease.

P11-10

Immunologic laboratory diagnostic tests for patients with chronic diarrhea

Mladenova T (1), Shentova R (2), Kyurkchiev D (1), Ivanova-Todorova E (1), Spassova Z (3), Altankova I (2)

(1) UHAT St. Ivan Rilski, Laboratory of Clinical Immunology, Sofia, Bulgaria
(2) UHATC Sofia, Pediatric Gastroenterology, Sofia, Bulgaria
(3) UHAT St. Ivan Rilski, Clinic of Gastroenterology, Sofia, Bulgaria

Corresponding author: cvety.mladenova@gmail.com

Background: Patients with chronic diarrhea are difficult to diagnose and treat. Along with the conventional clinical and instrumental tools for elucidating the diagnosis there are contemporary immunologic examination for immunomediated intestinal diseases such as celiac disease (CeD), Crohn`s disease (CD) and ulcerative colitis (UC). The aim of the study was to evaluate clinical significance of antibodies against deamidated gliadin peptide (anti-DGP), antibodies against Saccharomyces cerevisiae (ASCA), fecal calprotectin (FC) and fecal lactoferrin (FL) as diagnostic tools for establishing the diagnosis of CeD, CD and UC.

Materials and methods: We examined 137 patients – 37 with CD, 58 with CeD, 42 with UC. Sera and stool samples were tested. We also tested 25 healthy persons as control groups. The sera samples were evaluated with ELISA for anti-DGP antibodies (IgG) and ASCA (IgA), and the stool samples for FC and FL with a rapid Card test, lateral flow assay with Quantum Blue reader and ELISA for FC.

Results: Anti-DGP antibodies were positive in 100% of patients with CeD and in no one of UC and CD. ASCA were positive in 14,8% of CD and 25,9% of UC patients. FC was positive in 83% of UC, 87% of CD and 37.5% of CeD patients. FL was positive in 52% of UC and 54% of CD patients.

Conclusion: We found that these new immunologic markers are promising in diagnosis: anti-DGP is highly specific for CeD, ASCA are not very sensitive and discriminative for CD and UC. FL and FC are typical for active neutrophil inflammation.

P11-11

Polymeric forms of free light chains: two case reports

Illana Camara F, Castillo Perez C, Cardenas M, Arroyo M

Hospital Clínico San Carlos, Laboratory Medicine, Madrid, Spain

Corresponding author: carloscp2@hotmail.com

Background: Urinary Bence-Jones protein (BJP) refers to urinary excretion of monoclonal light chains. Light chains appear in the urine when the metabolizing capacity of nephron is exceeded. Negligible urinary BJP in light chain multiple myeloma patients due to polymeric forms have been described previously.

Materials and methods: Serum and 24-hour urine of two patients with multiple myeloma were studied. Capillary electrophoresis (Capillarys®) was used to detect and measure monoclonal protein (MP). Serum free light chains (FLC) were measured by immunonephelometry in an Immage 800 (Beckman Coulter®) analyzer. MP was identified by Immunofixation.

Results: Patient 1: A MP Lambda was detected in serum (13.2 g/L). Bence-Jones protein was identified and measured in urine (300 mg/24 hour). Serum κ and λ FLC were measured with a concentration of 10 mg/L and 18500 mg/L, respectively. Patient 2: Serum MP was detected. Immunofixation showed two different MPs, κ FLC and IgG κ. The concentration was 2.6 g/L and 9 g/L, respectively. No Bence-Jones proteinuria was detected. The concentration of κ and λ FLC was 9060 mg/L and 7 mg/L, respectively. Both patients had a normal renal function.

Conclusions: The presence of serum MP of FLC with absence or low levels of urinary BJP suggests a polymeric form of the MP with a large molecular size that prevents normal excretion by kidney. The difference between serum MP and FLC could be due to overestimation in the nephelometric measurement by the presence of polymeric forms of FLC that may have produced a more intense immunoprecipitation reaction.

P11-12

Eosinophilic cationic protein (ECP) and total IgE concentration in children with atopic diseases

Pancirov D (1), Ciprić M (1), Zukan I (2), Posavec M (1)

(1) "Dr. Ivo Pedišić" General Hospital, Department of Biochemistry and Hematology Diagnostics, Sisak, Croatia
(2) "Dr. Ivo Pedišić" General Hospital, Department of Pediatrics, Sisak, Croatia

Corresponding author: dolores.pancirov@sk.t-com.hr

Background: Eosinophilic cationic protein (ECP) is a basic protein located in the granules of eosinophil granulocytes and released during eosinophil activation. ECP serum levels are associated with the intensity of allergic inflammation. Elevated total IgE concentration is common to all atopic diseases. The aim of the study was to compare ECP and total IgE concentration in sera of patients with asthma, allergic rhinitis and atopic dermatitis, their values with values obtained from healthy children

and to determine the relationship between these analytes in the mentioned groups of patients.

Materials and methods:The study included 43 children with asthma, 20 with allergic rhinitis, 20 with atopic dermatitis and 30 healthy children as controls. ECP and total IgE serum values were determined using an automated fluorescence enzyme immunoassay (FEIA) on an UniCAP R 100 immunoanalyzer.

Results: Although the ECP concentration median was highest in the group of asthmatic patients (15.3 µg/L) and lowest in the group of atopic dermatitis patients (10.4 µg/L), there was no significant difference among the groups of patients, while total IgE concentration was higher in the groups of patients with asthma and allergic rhinitis ($P < 0.001$). ECP and total IgE concentrations were significantly higher in patients with asthma ($P < 0.001$ for both) and allergic rhinitis ($P = 0.018$; $P < 0.001$) compared to controls. Weak positive correlation between these analytes was found in asthmatic patients ($r = 0.478$, $P = 0.001$).

Conclusion: The results indicate that ECP values can be used as a marker of inflammation in asthmatic patients and those with allergic rhinitis. ECP and total IgE concentrations were weakly correlated only in asthmatic patients.

P12 – Kidney diseases

P12-01

The performance of compensated serum creatinine in pediatric samples

Aralica M, Matica J

Rijeka University Hospital Center, Clinical Institute of Laboratory Diagnostis, Rijeka, Croatia

Corresponding author: merica.aralica@gmail.com

Background: According to current recommendation serum creatinine measurement in adults should be performed by compensated Jaffe method. The enzymatic method is recommended for pediatric population but high commercial price is limiting factor for its implementation in routine practice. We estimated proportion of compensated serum creatinine concentration below measuring range in children; following to method comparison analysis of compensate Jaffe *vs*. enzymatic creatinine method.

Materials and methods: A total of 58 pediatric serum samples were included in the study (median age 44 months, age range 2 days-18 years). The measurement of creatinine by enzymatic method (measuring range 0-2700 µmol/L) was done on the Cobas c311 analyzer (Roche Diagnostic) and by compensated Jaffe method (measuring range 18-2200 µmol/L) on the Olympus AU400 analyzer (Beckman Coulter).

Results: In 36 out of 58 samples (group 1, median age 66 months, age range 2 days-18 years) concentrations of compensated creatinine were in measuring range (median 34 µmol/L, range 18-195 µmol/L) but it was not a case in 22 out of 58 samples (group 2, median age 12 months, age range 15 days-6 years) with compensated creatinine concentrations below measuring range. There was a significant difference between two groups regarding age (t-test, $P < 0.001$). The Passing and Bablok regression analysis showed ($N = 36$) intercept -4.42 (95%CI 6.03 to -3.00), slope 1.02 (95%CI 1.00 to 1.06); $r = 0.99$; range tested 18-195 µmol/L.

Conclusion: A high proportion of creatinine concentrations under measuring range was unacceptable, concerning younger children. Method comparison analysis revealed underestimation of pediatric serum creatinine by compensated Jaffe method.

P12-02

The impact of different methods of creatinine measurements on MELD scoring system

Perkov S (1), Flegar-Meštrić Z (1), Ožvald I (1), Kocman B (2), Čolić-Cvrlje V (3), Čakalo Lj (3)

(1) University Hospital Merkur, Institute of Clinical Chemistry and Laboratory Medicine, Zagreb, Croatia
(2) University Hospital Merkur, Department of Abdominal Surgery and Organ Transplantation, Department of Surgery, Zagreb, Croatia
(3) University Hospital Merkur, Department of Internal Medicine, Zagreb, Croatia

Corresponding author: sonja.perkov@gmail.com

Background: The Model for End-Stage Liver Disease (MELD) scoring system is used to prioritize patients for liver transplantation by their disease severity based on three laboratory parameters: serum creatinine and bilirubin concentrations and INR prothrombin ratio. The aim of this study was to investigate impact of two different creatinine methods on MELD score.

Materials and methods: We assessed 40 blood samples obtained from 32 patients listed for liver transplantation. Serum creatinine concentrations was measured by the kinetic Jaffe method (Beckman Coulter OSR6178) traceable to NIST SRM 909b level 2 and enzymatic method (Beckman Coulter OSR61204) traceable to the IDMS method and SRM 967. The MELD score was calculated according to the formula currently in use by Eurotransplant. Patients are stratified in a descening order starting with the highest MELD.

Results: There was a significant difference in serum creatinine among Jaffe and enzymatic methods: median 93 (range 67-744) *vs*. 73,5 (range 49-747) µmol/L, respectively. The variation in creatinine measurements resulted in differences up to 2 points in a single patients. When the enzymatic methods was used instead of Jaffe methods, MELD scores were unchanged in 23 cases wheras in 17 cases MELD scores decreased by 1-2 point.

Conclusion: Observed variability in the assessment of the MELD score due to methodological variation in serum creatinine measurement may not significantly alter prognosis or have clinical consequences but may affect prioritization for liver transplantation. For this reason standardization of creatinine measurements using specific enzymatic method traceable to SRM 967 is of most importance.

P12-03

"Screening" for chronic renal disease in a Portuguese population

Cachapuz I (1), Branco A (2), Silva S (2), Alves V (1), Costa M (2)

(1) Matosinhos Local Health Unit, Clinical Pathology Service, Oporto, Portugal, Portugal
(2) Matosinhos Local Health Unit, Nephrology Service, Oporto, Portugal

Corresponding author: cachapuz.isabel@gmail.com

Background: According to the "Chronic Renal Disease (CRD) national registration" in terms of renal replacement therapy, the incidence of patients on dialysis has been increasing in Portugal, in the last decade. However, there is no precise information about prevalence of CRD at national level. We have conducted a study to assess the extent of the problem in the population of Matosinhos Local Healthcare Unit (ULSM).

Materials and methods: Evaluation of glomerular filtration rate (GFR) with MDRD-4 formula, in ULSM population with scheduled medical consultation between Feb/2010 and Feb 2011. Patients from hospitalization and emergency were excluded and acute renal failure cases were avoided. Patients with at least two 60 mL/h/m^2 measuring of GFR in a 3 months minimum interval.

Results: Evaluation of renal function of 44869 patients (29 were excluded due to incomplete data), average: 54 years (minimum 12; maximum 99); M: 41.9% / F: 58.1%; 3949 had GFR < 60 in at least one measuring. 1547 patients met the CRD criteria, which corresponds to 3.4% prevalence. From those 74.6% are in stage 3 (prevalence = 2.57%); 20.6% in stage 4 (prevalence = 0.71%) and 4.6% in stage 5 (prevalence = 0.16%). 38.4% of the CRD population are diabetics.

The global prevalence of CRD seems slightly inferior to the described in European studies. Diabetes mellitus is still a major risk factor for these individuals.

Conclusions: The introduction of the analytical protocol for calculating GFR proved to be a good CRD screening method, like in most European countries. It helps prevent CRD and reduce the cardiovascular risk.

P12-04

Osteoporosis and kidney impairment in postmenopausal women

Pavić M (1), Milevoj Kopčinović L (1), Car D (2), Nikolić T (3)

(1) Medical School University Hospital Sestre Milosrdnice, Zagreb, Croatia, Clinical Unit of Medical Biochemistry in Traumatology and Orthopedics, University Department of Chemistry, Zagreb, Croatia
(2) Medical School University Hospital Sestre Milosrdnice, Zagreb, Croatia, Clinical Unit of Internal Medicine, University Hospital of Traumatology, Zagreb, Croatia
(3) Medical School University Hospital Sestre Milosrdnice, Zagreb, Croatia, Trauma Rehabilitation Unit, University Department of Rheumatology, Physical Medicine and Rehabilitation, Zagreb, Croatia

Corresponding author: marina.pavic@kbcsm.hr

Background: Both osteoporosis and kidney impairment become more prevalent with age. The aim of this study was to investigate the prevalence of compromised kidney function in women with osteopenia and osteoporosis, and to assess the agreement between GFR estimated with creatinine clearance (CrCl) and the Cockcroft-Gault equation (CG).

Materials and methods: We studied postmenopausal women, divided according to T-scores into two groups: normal/osteopenic and osteoporotic. They were further divided, using CrCl and CG, into groups depending on chronic kidney disease. Serum calcium, phosphorus and creatinine, 24hour-urine calcium and creatinine were determined. Estimated GFRs were calculated using the CrCl and CG. Informations about age, height, weight were collected.

Results: 190 postmenopausal women (median age 67 (45-88) years) were divided into subgroups: normal/osteopenia (N = 143; 75%) and osteoporosis (N = 47; 25%). Differences were found comparing age, weight, urine creatinine and eGFR with the CG. The prevalence of kidney impairment (GFR < 60 mL/min/1.73 m^2) according to CrCl and CG in normal/osteopenic women was 17% and 19%, respectively; and in the osteoporosis group 34% and 36%, respectively. The prevalence was higher in the osteoporosis group (P = 0.029 for CrCl; P = 0.025 for CG). Weighted kappa between CrCl and CG was 0.500, 0.483 and 0.487 in the overall, normal/osteopenia and osteoporosis subgroup, respectively.

Conclusions: Our results showed substantial prevalence of kidney impairment in postmenopausal women with osteoporosis. This must be taken into account when considering the prescription of medications with kidney clearance (like bisphosphonates). The kappa statistics classified the agreement between CrCl and CG as moderate.

P12-05

Iron content of serum ferritin: a biomarker of iron storage

Mujagic R (1), Obuljen J (2), Crnokrak S (1)

(1) General Hospital Pula, Clinical laboratory, Pula, Croatia
(2) Children's Hospital Zagreb, Biochemical-Hematology Laboratory, Zagreb, Croatia

Corresponding author: renat.mujagic@pu.t-com.hr

Introduction: Ferritin is an iron-storage protein. The serum ferritin concentration reflects the amount of iron in the human body. Serum ferritin concentration is non-specifically elevated in the presence of infection, inflammation, liver disease and malignancies. Iron content of serum ferritin (ICF) isn't influenced by inflammation and ICF represents an iron status biomarker regardless of inflammation. Thereafter, ICF can be confidently used to assess a functional iron deficiency in patients undergoing hemodialysis.

Materials and methods: Fifty-five hemodialysed patients that had been treated with Ferrlecit and/

or erythropoietin past three months were included in this clinical trial. Ferritin, transferrin, serum non-ferritin iron and immaturity reticulocytes fraction index (IRF) were measured by standard methods. Total iron concentration was measured by Inductively Coupled Plasma-Mass Spectrometry (ICP-MS). ICF was calculated from total iron, non-ferritin iron and ferritin concentration.

Results: There were significant positive association between logarithm-transformed ICF with IRF (rP = 0.377; P = 0.005) and transferrin (rP = 0.555; P < 0.001). ICF median (14.1 µmol/mg) was below reference range.

Conclusion: Transferrin concentration firstly depends on nutritional status, and ferritin concentration firstly depends on inflammation rather than iron status. Concussively, ferritin and/or transferrin don't represent confidential biochemical predictors of iron deficiency in hemodialysed patients. Regardless of inflammation, ICF can confidently predict functional iron availability for erythropoiesis. ICF represents clinically relevant biomarker which reflects the true iron needs and can be safely used to optimize iron supplementation for correcting iron deficiency in patients undergoing hemodialysis.

P12-06

Nitric oxide and homocysteine in patients with chronic kidney disease

Mikulić I (1), Petrik J (2), Čepelak I (2), Tomić M (3), Baković V (3), Mikulić V (1)

(1) Mostar University Hospital, Department of Laboratory Diagnosis, Široki Brijeg, Bosnia and Herzegovina
(2) Faculty of Pharmacy and Biochemistry, Department of Medical Biochemistry and Hematology, Zagreb, Croatia
(3) Mostar University Hospital, Department of Nephrology and Hemodialysis, Clinic for Internal Diseases, Mostar, Bosnia and Herzegovina

Corresponding author: ivankacolak@yahoo.com

Background: Nitric oxide (NO) and homocycteine have an important role in the complex pathogenesis of chronic kidney disease (CKD).

Material and methods: The levels of NO (spectrophotometric assay, Griess reaction) and homocysteine (chemiluminescence microparticle immunoassay) were determined in plasma/serum and/or urine of patients with CKD (N = 101) and of the control subjects (N = 51). The total number of patients with CKD was divided into subgroups according to: a) the primary cause of impaired renal function, b) the National Kidney Foundation guidelines, and c) the intensity of proteinuria.

Results: The results show that serum concentrations of NO (median 9.20 µmol/L), and homocysteine in plasma (median 16.95 µmol/L) in patients with CKD was significantly higher (P = 0.009, P < 0.001) compared with the control group (median 7.27 µmol/L and 13.04 µmol/L, respectively). The concentration of homocysteine in plasma showed a relatively good diagnostic sensitivity (60.0 to 89.5%) and diagnostic specificity (63.4 to 90.2%) in distinguishing between groups of patients with CKD and a group of control subjects.

Conclusions: Results of logistic regression showed that the increase of both NO level and homocysteine level for 2.72 µmol/L raises the possibility for development of CKD (2 times for NO: OR = 2.021, or 6.5 times for homocysteine: OR = 6.512). Moreover, the increase of NO level in serum and increase of homocysteine level in plasma is a risk factor for progression to higher stages of CKD (P = 0.002 and P < 0.05, respectively).

P12-07

Estimated glomerular filtration rate, hypertension and blood mercury in a hospital working population

Diaz Diaz A, Morales C, Trasobares E, Castillo Perez C, Fuentes M, González-Estecha M

Hospital Clínico San Carlos, Laboratory Medicine, Madrid, Spain

Corresponding author: avellaneda.diaz.diaz@gmail.com

Background: Blood mercury has been associated with nephrotoxicity and cardiovascular disease among the general population. The aim of this study is to evaluate the association between blood mercury concentrations and estimated glomerular filtration rate (eGFR) and hypertension in a hospital working population.

Material and methods: We recruited 395 employees (64 men and 331 women) who were given the EMA® exposure questionnaire. Blood mercury concentration (µg/L) was measured by atomic absorption spectrometry and thermal decomposition amalgamation. Serum selenium concentration (µg/L) was measured by electrothermal atomic absorption spectrometry. eGFR was assessed using the abbreviated MDRD formula (Levey et al. 2000).

Results: The median of blood mercury was 8.00 µg/L (IQR: 5.20-11.60) and the mean of eGFR was 74.44 mL/min/l.73 m^2 (SD: 10.29). A statistically significant correlation was found between blood mercury and the eGFR (r = -0.127, P = 0.014). This significant correlation was observed in women (r = -0.109; P = 0.05) but not in men. In order to explore possible hidden kidney disease we used a cutoff of 60 mL/min/l.73 m^2 for eGFR and a cutoff of 5.8 µg/L for mercury and no significant differences were observed. A positive correlation between eGFR and selenium/mercury ratio was found (r = 0.11; P = 0.034). This significant correlation was seen in men (r = 0.255; P = 0.049) but not in women. No differences were observed between hypertension and blood mercury or with the selenium/mercury ratio.

Conclusions: The association between eGFR and mercury is different when considering the selenium status. It is necessary to consider the interactions between these elements in order to evaluate mercury toxicity.

P12-08

Cadmium, estimated glomerular filtration rate and hypertension in a hospital working population

Diaz Diaz A, González-Estecha M, Castillo Perez C, Morales C, Fuentes M, Arroyo M

Hospital Clínico San Carlos, Laboratory Medicine, Madrid, Spain

Corresponding author: avellaneda.diaz.diaz@gmail.com

Background: Low cadmium levels (0.43 µg/L) have been associated with nephrotoxicity and cardiosvascular disease among the general population. The aim of this study is to evaluate the association between blood cadmium concentrations with estimated glomerular filtration rate (eGFR) and hypertension in a hospital working population.

Material and methods: We recruited 395 employees (64 men and 331 women) who were given the PESA® exposure questionnaire. Blood cadmium was measured by electrothermal atomic absorption spectrometry. eGFR was assessed using the abbreviated MDRD formula (Levey et al. 2000): eGFR [mL/min/l.73 m^2] = 186 × (Serum creatinine [mg/dL])-1.154 × (Age [y]) 0.203 (× 0.742 if female).

Results: The median of blood cadmium was 0.29 µg/L (IQR:0.18-0.50) and the mean of eGFR was 74.44 mL/min/l.73 m^2 (SD:10.29). A statistically significant correlation was found between blood cadmium and the eGFR (r = -0.176, P = 0.001). This significant correlation was also observed in men (r = -0.301; P = 0.019) and women (r = -0.147; P = 0.009). In order to explore possible hidden kidney disease we used an eGFR cutoff of 60 mL/min/l.73 m^2 and a cadmium cutoff of 0.43 µg/L and no significant differences were found. In connection

with hypertension, we found no significant differences between blood cadmium concentrations and hypertension were found.

Conclusions: The association found between eGFR and cadmium levels in men as well as in women supports the role of cadmium in kidney disease. However, using a cutoff of 60 mL/min/l.73 m^2 we cannot confirm this correlation, probably due to the low number of events under the cutoff used.

P12-09

uNGAL in deceased donors as a marker of early kidney transplant injury

Kalachyk A (1), Oganova E (2), Naderi U (3), Sadovski D (1), Dolgolikova A (1)

(1) National Organs and Tissues Transplant Center, Minsk, Belarus, Transplant Department, Minsk, Belarus
(2) National Organs and Tissues Transplant Center, Minsk, Belarus, Clinical Laboratory, Minsk, Belarus
(3) National Organs and Tissues Transplant Center, Minsk, Belarus, Department of coordination, Minsk, Belarus

Corresponding author: elenaog@newmail.ru

Background: The standard approach to the selection of deceased donors by assessment of urine output and serum creatinine levels do not allow to predict delayed graft function (DGF) or slow GF.

Materials and methods: We analyzed the association between the concentration of uNGAL and renal graft function (RGF) in early postoperative period in deceased donors with brain death. Urine was collected in 25 deceased donors and analyzed using Architect.

Results: DGF was diagnosed in 10% and SGF in 6% kidney recipients. All patients were devided into 2 groups according to uNGAL concentrations. 1st group - 26 recipients that obtained renal graft from deceased donors with low NGAL concentration (≤ 18 ng/mL), the 2nd - with high NGAL(≥ 18 ng/mL). There were no significant differences in mean donor age, sex ratio (male/female), time of cold storage and cause of death in these groups. A comparative analysis showed that delayed or SGF occured in the 1st group in 11.5% (3/26) patients, and in the 2nd group-21.7% (5/23) patients respectively. RGF was significantly better in the 1st recipients group: creatinine 117.4 ± 25.5 mmol/L, eGFR using MDRD 78.4 ± 29.5 mL/min, the 2nd recipients groups: creatinine 141.4 ± 72.4 mmol/L and eGFR-68.9 ± 20.1 mL/min ($P < 0.05$) 1 month after the transplantation.

Conclusion: The study showed that uNGAL level in deceased donor with brain death can be used as a biomarker for prediction of RGF in early postoperative period.

P12-10

Low-grade inflammation and iron metabolism in chronic kidney disease (CKD) patients

Tandara L, Salamunić I, Gugo K, Bilopavlovic N, Pauković-Sekulić B, Tandara M

University Hospital Centre Split, Department of Medical Laboratory Diagnosis, Split, Croatia

Corresponding author: leida.tandara@gmail.com

Background: Patients with CKD are exposed to persistent low-grade inflammation. Increased level of proinflammatory cytokines cause cellular iron retention and repress iron efflux from sites of main iron flow into the blood reducing thus iron availability and contributing to development of anemia in CKD patients. This study aimed to assess relationship between indicators of inflammation and routine biochemical markers of iron metabolism as well as hematological indicators of iron availability in group of HD patients with low-grade inflammation (defined as CRP < 15 mg/L, without clinical manifestation of inflammation).

Materials and methods: The study was conducted in 45 HD patients and 21 healthy subjects. Biochemical markers (serum iron, transferrin, transferrin saturation, ferritin), haematological indexes (%Hypo and CHr) were determined by routine lab-

oratory methods. As inflammatory markers, CRP and hsIL-6 were determined.

Results: Levels of serum CRP [2.9 (0.9-10.9) *vs*. 0.8 (0.4-3.2) mg/L] and IL-6 [5.03 (1.69-10.5 *vs*. 1.20 (0.14-10.55) ng/L] were significantly higher in HD patients compared with control group ($P < 0.05$). We found a statistically significant ($P < 0.05$) lower level of serum iron, transferrin, transferrin saturation and higher level of ferritin and %Hypo but there were no significant correlations between CRP/IL-6 and biochemical markers of iron status or hematological indexes in HD group.

Conclusions: Parameters of iron metabolism are changed but inflammation indicators do not correlate with parameters of iron metabolism in our studied group. Since levels of serum inflammatory markers are subjected to a substantial variability over time repeated versus single measurements in a future studies could give a more valuable information about examined relationship.

P12-11

Cystatin C in early diagnosis of contrast-induced acute kidney injury

Vavilova T, Klyusova S, Gaykovaya L

North-West State Medical University named after Mechnikov, Laboratory Department, Saint-Petersburg, Russia (Russian Fed.)

Corresponding author: vtv.lab@rambler.ru

Objective: Contrast-induced acute kidney injury (AKI) may take place in some patients after percutaneous coronary interventions and diagnostic procedures. Early diagnosis allows beginning the treatment in time and preventing severe renal insufficiency. Recent data showed cystatin C (CysC) as useful marker in AKI, but less information is about the contrast-induced one.

Materials and methods: The level of serum creatinine (Cr) and CysC was measured before and after 18-20 h of contrast administration in 30 patients, admitted for coronary angiography and/or balloon angioplasty in university clinic. All patients had risk factors but not confirmed kidney injury; 28 men and 2 women, 63.0 ± 9.9 ears old. CysC was measured by immunoturbidimetry method (KONELAB-20 analyser, Alfresa kit, Japan). Glomerular filtration rate (GFR) was calculated by MDRD and the equation Levey for cystatin C.

Results: The average increase in cystatin C level in 18-20 hours was 21.0%, and in serum creatinine - 5.5% ($P < 0.05$). The cystatin C sensitivity was found to be 94%, and specificity - 69% for ACI. Decreasing of the GFRCysC and GFRMDRD also showed significant differences: from 92.4 ± 36.8 to 73.1 ± 26.6 mL/min/1,73 m^2 (20.9%); and from 81.3 ± 19.1 to 77.2 ± 20.7 mL/min/1,73 m^2 (5.0%), respectively ($P < 0.05$).

Conclusion: Cystatin C proved to be an early marker in interventional cardiology and may be used for the diagnosis of contrast-induced acute kidney injury.

P12-12

Protein:Creatinine ratio in spot urines to predict proteinuria in chronic kidney disease

Shrestha R (1), Gyawali P (2), Neopane P (3), Jha B (2)

(1) Nepal Medical College and Teaching Hospital, Clinical Biochemistry, Kathmandu, Nepal
(2) Institute of Medicine, TU Teaching Hospital, Biochemistry, Kathmandu, Nepal
(3) Medicare National Hospital, Laboratory Medicine, Kathmandu, Nepal

Corresponding author: cl.biochem@gmail.com

Background: The measurement of protein in 24-hr urine collection has been regarded as the gold standard. Spot urine protein:creatinine ratio (PCR) have been widely used as alternative to 24h urine protein (UTP).

Objective: The aim of this study was to examine the ability of PCR to predict urinary 24h protein loss in Nepalese patients with CKD.

Materials and methods: This Study comprises 933 CKD patients (178 had stage 1 CKD, 230, 222, 158, and 145 patients with stage 2, 3, 4, and 5 re-

spectively). Patients were instructed to collect 24h urine and spot urine, both were subjected for determination of UTP and spot PCR.

Results: 24.3%, 25%, 46.8%, 75.7% and 90.1% of stage 1, 2, 3, 4 and 5 CKD respectively had proteinuria as determined by UTP (> 150 mg/day). There was good correlation between UTP and PCR, with correlation coefficients(r) of 0.923. Receiver operator characteristic (ROC) curve analysis showed PCR to be a good predictor of proteinuria, with area of 0.973 (95% CI; 0.961-0.985, P < 0.001). At PCR cutoff of 20 mg/mmol sensitivity of 93% and specificity of 92% can be achieved. The optimal cutoff varies among stage of CKD. The optimal cutoff of PCR is 13.2 mg/mmol in stage 1, 15.1 20 mmol in stage 2, 20.5 mg/mmol in stage 3, 26.1 mg/mmol in stage 4 and 33 mg/mmol in stage 5.

Conclusions: Spot PCR can be a good alternative to 24 hour UTP. By careful choice of cutoffs, PCR can be used in patients with CKD to identify significant proteinuria.

P12-13

Sensitivity and specificity of NGAL in acute pyelonephritis

Mandić S (1), Arambašić J (2), Horvat V (1), Mandić D (3), Šerić V (1)

(1) University Medical Center Osijek, Department of Clinical Laboratory Diagnostics, Osijek, Croatia
(2) University Medical Center Osijek, Pediatric Clinic, Osijek, Croatia
(3) Institute of Public Health for Osijek-Baranya Country, Health Ecology Department, Osijek, Croatia

Corresponding author: mandic.sanja@gmail.com

Background: Neutrophil gelatinase-associated lipocalin (NGAL) is a small protein expressed in renal tubules where it is considerably induced in ishemic or nephrotoxic injury. A single urine NGAL measurement is proposed test for distinguish acute kidney injury. However, single urine can sometimes be concentrated or diluted, which can influence results of measurements.

Materials and methods: We have analyzed 90 children under 5 years of age, which were admitted to the Pediatric Clinic. 62 of them had acute pyelonephritis, while 28, who represented controls, had cystitis or fever of other etiology. Routine laboratory analyses (CBC, CRP and urine analysis), urine culture, kidney ultrasound and static scintigraphy were also done. Urine NGAL was measured using CMIA method (ARHITECT i1000, ABBOT). We have compared single NGAL concentrations with mg NGAL/g creatinine in same patient. Statistical calculation was performed using Mann-Whitney rank sum test and ROC analyses.

Results: At a cut-off value of 29.1 ng/ml NGAL, sensitivity and specificity for detecting acute pyelonephritis was 0.964 (95% CI, 0.901 to 0.992), while at cut-off value of 120.1 mg NGAL/g creatinine sensitivity and specificity was 0.988 (95% CI, 0.938 to 1.000). Mann-Whitney rank sum test also showed significant difference in median values between the two groups for both measurements (P < 0.001).

Conclusion: Although the values of NGAL / g creatinine were a bit superior, there is no statistical significant difference between measurements.

P12-14

Assessment of the new CKD-EPI formula to estimate CKD in hospitalized patients

Fernandez Rodriguez E (1), Garcia Alonso S (1), Fernandez Garcia E (2)

(1) Cabuenes Hospital, Biochemistry, Gijon, Spain
(2) Central Hospital, Intensive Care Unit, Oviedo, Spain

Corresponding author: eloyfr@gmail.com

Background:A recent report by the CKD-EPI group describes a new equation to estimate the GFR. The CKD-EPI equation improved the accuracy and precision of results of the current first-choicec MDRD IDMS formula, specially for GFR > 60 mL/min/1.73m^2. A high percentage (28.3%) of hospitalized patients in Spain have deteriorated renal function stages 3-5 as measured by MDRD 4 formula.

Materials and methods:The goal of our study was to compare the estimated GFR by using the

new equation CKD-EPI with MDRD 4 in a wide cohort of hospitalized patients (14,658 adults) and to analyze the impact of the new CKD-EPI formula on the satging of patients with CKD.

Results: The concordance correlation coefficient beetween both formulas was 0.9949 (95% CI: 0.9947 to 0.9951). The distribution of KDOQI stages were: CKD-EPI (1 + 2, 69.9%; 3a, 14.9%; 3b, 9.4%; 4, 4.3%; 5, 1.5%),MDRD (1 + 2, 72.7%; 3a, 14.9%; 3b, 7.7%; 4, 3.4%; 5, 1.2%). Weighted Kappa statistics was 0.861 (very good agreement). Overall, CKD-EPI detected an additional 2.8% of patients with GFR < 60 mL/min/1.73m^2.

Conclusions: CKD-EPI equation reclasified an additional 2.8% of patients to stages of worse GFR

P13 - Lung, liver and gastrointestinal diseases

P13-01

Serum copper concentrations and cardiomyopathy in cystic fibrosis patients

Castillo Perez C (1), González-Estecha M (1), Morales C (1), Ruiz L (2), Díaz Díaz A (1), Segovia J (2)

(1) Hospital Clínico San Carlos, Laboratory Medicine, Madrid, Spain
(2) Hospital Puerta de Hierro Majadahonda, Cardiology, Madrid, Spain

Corresponding author: carloscp2@hotmail.com

Background: Copper deficiency has been reported in cardiomyopathy and may occur in patients with intestinal malabsorption, as occurs in cystic fibrosis (CF). The aim of this multicenter study is to evaluate copper in CF patients, who have a high prevalence of cardiomyopathy.

Materials and methods: We studied 123 adult CF patients (63 male and 60 female) with a mean age of 31 (SD: 8.90). Serum copper concentrations were measured using flame atomic absorption spectrometry. The concentration of serum ceruloplasmine was measured by immunonephelometry. In addition, we estimated the Cu/Ceruloplasmine ratio. The left ventricular ejection fraction (LVEF) was determined by a Philips IE33 Echocardiogram. We defined systolic dysfunction as an LVEF less than 55% (Simpson's method).

Results: The mean copper concentration was 131.8 µg/dL (SD: 37.7). The mean serum ceruloplasmine was 34.00 mg/dL (SD: 9.1). The mean Cu/Ceruloplasmine ratio was 3.9 (SD: 0.4). No correlation was found between total copper and LVEF or between the Cu/ceruloplasmine ratio and LVEF. However, upon considering patients with an LVEF under the cutoff of 55 % we found a lower serum copper concentration (117.8 µg/dL SD:18.4 *vs*. 132.6 µg/dL SD: 38.3), although this difference was not statistically significant.

Conclusion: In spite of the malabsorption associated with CF, we did not observe copper deficiency in this population. Since we found a decrease in copper concentrations in patients with lower LVEF, more studies should be performed with a greater sample size in order to clarify the role that copper may play in the cardiomyopathy of CF patients.

P13-02

Lead and cadmium in cystic fibrosis

Castillo Perez C (1), González-Estecha M (1), Díaz Díaz A (1), Ruiz L (2), Maíz L (3), Segovia J (2)

(1) Hospital Clínico San Carlos, Laboratory Medicine, Madrid, Spain
(2) Hospital Puerta de Hierro Majadahonda, Cardiology, Madrid, Spain
(3) Hospital Ramon y Cajal, Neumology, Madrid, Spain

Corresponding author: carloscp2@hotmail.com

Background: Exposure to lead and cadmium is a public health problem due to the broad exposure to these toxic substances among the general population, and in recent years they have been associated with an increased cardiovascular risk. The objective of this multicenter study is to determine blood lead and cadmium concentrations in a population of unselected patients diagnosed with

cystic fibrosis (CF), who have a higher prevalence of cardiomyopathy than expected.

Material and methods: We studied 123 adult CF patients (63 male and 60 female) with a mean age of 31 (SD: 8.90). The blood lead (µg/dL) and cadmium (µg/L) concentrations were measured by electrothermal atomic absorption spectrometry with Zeeman background correction in a Perkin-Elmer spectrometer. The left ventricular ejection fraction (LVEF) was determined by a Philips IE33 echocardiogram. We defined systolic dysfunction as an LVEF less than 55% (Simpson's method).

Results: The median of lead was 0.80 µg/dL (IQR: 0.48-1.13). Blood lead percentiles (5, 25, 50, 75, 95) were: 0.07, 0.48, 0.80, 1.13, 1.91 µg/dL respectively. Eighty per cent of the patients had blood cadmium concentrations below the detection limit (0. 07 µg/L). Blood cadmium percentiles (75, 90, 95) were: 0.07, 0.3, 0.7 µg/L. No significant differences were found between lead and cadmium levels and LVEF.

Conclusion: The concentrations of blood lead in this population are low and similar to the reference values for this age group in Spain. Most of these patients showed blood cadmium levels below the detection limit probably because of their low level of smoking.

13-03

Interaction of blood mercury with essential trace elements in a cystic fibrosis population

Morales C (1), González-Estecha M (1), Castillo Perez C (1), Ruiz L (2), Prados C (3), Segovia J (2)

(1) Hospital Clínico San Carlos, Laboratory Medicine, Madrid, Spain
(2) Hospital Puerta de Hierro Majadahonda, Cardiology, Madrid, Spain
(3) Hospital Universitario La Paz, Neumology, Madrid, Spain

Corresponding author: carloscp2@hotmail.com

Background: Exposure to mercury is a public health issue. Cystic fibrosis is characterized by an obstructive pulmonary pattern and a pancreatic exocrine deficiency, frequently associated with malabsorption. We studied the relationship between mercury, copper, zinc and selenium in this multicenter population.

Materials and methods: We studied 123 adult CF patients (63 male and 60 female) with a mean age of 31 (SD: 8.90). Serum copper (µg/dL) and zinc (µg/dL) were measured using flame atomic absorption spectrometry. Serum selenium (µg/L) was measured using electrothermal atomic absorption spectrometry. Blood mercury (µg/L) was measured by atomic absorption spectrometry and thermal decomposition amalgamation.

Results: The median of blood mercury was 5.7 µg/L (IQR: 2.9-9.6). Forty eight per cent of the patients had blood mercury levels higher than the levels established by the EPA (5.8 µg/L). The mean of serum copper was 131.8 µg/dL (SD: 37.7). The mean of serum zinc was 86.9 µg/dL (SD: 13.3) and for selenium was 71.9 µg/L (SD: 14.8). A negative correlation was found between mercury and zinc ($r = -0.125$) which was not statistically significant; no correlation was observed between mercury and copper. A positive correlation was found between mercury and selenium ($r = 0.308$, $P < 0.001$). This correlation was observed in both patients with blood mercury levels under 5.8 µg/L ($r = 0.304$, $P = 0.012$) and in those with levels above 5.8 µg/L ($r = 0.333$, $P = 0.007$).

Conclusion: We found high mercury levels in patients with CF. Further studies are desirable to investigate the interactions with essential trace elements and different compounds of the diet which could prevent mercury toxicity.

P13-04

Selenium and mercury and the left ventricular ejection fraction in adult cystic fibrosis patients

González-Estecha M (1), Ruiz L (2), Castillo Perez C (1), Morales C (1), Díaz Díaz A (1), Segovia J (2)

(1) Hospital Clínico San Carlos, Laboratory Medicine, Madrid, Spain
(2) Hospital Puerta de Hierro Majadahonda, Cardiology, Madrid, Spain

Corresponding author: carloscp2@hotmail.com

Background: Cystic fibrosis (CF) is frequently associated with malabsorption. Certain studies have described a rare form of cardiomyopathy (CMP), similar to the one seen in Keshan's disease. The aim of this multicenter study is to measure serum selenium and blood mercury and their relation with cardiomyopathy in CF patients.

Materials and methods: We studied 123 adult CF patients (63 male and 60 female) with a mean age of 31 (SD: 8.90). Blood mercury concentration (µg/L) was measured by atomic absorption spectrometry and thermal decomposition amalgamation. Serum selenium concentration (µg/L) was measured by electrothermal atomic absorption spectrometry. The Left Ventricular Ejection Fraction (LVEF) was determined by echocardiography. We defined systolic dysfunction as an LVEF of less than 55% (Simpson's method).

Results: The patients with serum selenium concentrations below 60 µg/L had a lower mean LVEF (58.86% SD: 12.10) than the ones with serum selenium concentration above 60 µg/L (65.21% SD: 6.23) and this difference was statistically significant (P = 0.001). The difference between these means (B = 6.36; P < 0.001 CI 95%: 2.76-9.95) can be explained in 9.4% by selenium (R^2 = 0.094; P < 0.001) and 9.8% can be explained by the selenium/mercury ratio (R^2 = 0.098; P < 0.002). However, once adjusted for mercury this last difference can be explained only by selenium (B = 6.45; P = 0.001 CI 95%: 2.58-10.33). No significant differences were found between mean LVEF and blood mercury levels (cutoff of 5.8 µg/L) and the selenium/mercury ratio (cutoff of 10.34).

Conclusion: Studying the association between mercury, selenium and LVEF, we observed that only low selenium concentrations are related to alterations in LVEF.

P13-05

Monitoring faecal occult blood test positivity in the NHS Bowel Cancer Screening Programme

Halloran S, Burtonwood C, Butler P, Young M

NHS Bowel Cancer Screening Programme, Southern Hub, Guildford, United Kingdom

Corresponding author: s.halloran@nhs.net

Background: The guaiac-based faecal occult blood test kit used by the NHS Bowel Cancer Screening Programme (BCSP) relies on subjective visual assessment to determine positivity. BCSP participants apply two samples from three separate bowel motions to each of six test kit windows lined with filter paper impregnated with guaiac. Test kits are returned to the Hub for analysis where development of an unstable blue-green colour in response to a peroxide developer indicates the presence of haemoglobin. Individuals who test positive are referred for further investigation, usually colonoscopy. The subjective nature of the test kit reading and possible inaccuracies have consequences for the national programme, participants and colonoscopy services.

Materials and methods: Test kit readers are tested for colour blindness and visual acuity when they start work in the Hub. The percentage of positive test spots is recorded weekly for readers completing > 100 kits. The acceptable spot positivity range (1-4%) is based on an approximation of ± 2 standard deviations from the rolling six-month mean percentage positivity for all Hub staff.

Results: Since monitoring began in 2010 there has been a reduction in reader imprecision and outliers. New staff attend training sessions to learn about

the concept of reader positivity and the interventions that may be put in place if their positivity falls outside the acceptable range and supervised refresher training for all staff is conducted annually.

Conclusion: Monitoring of test kit readers' performance is essential whilst adoption of an automated immunochemical test analysis is the long-term solution.

P13-06

The NHS Bowel Cancer Screening Programme, Southern Hub – screening activity and outcomes

Halloran S, Seaman H, Young M

NHS Bowel Cancer Screening Programme, Southern Hub, Guildford, United Kingdom

Corresponding author: s.halloran@nhs.net

Background: The NHS Bowel Cancer Screening Programme (BCSP) in England invites individuals aged 60-74 years to be screened every two years. The BCSP Southern Hub serves a population of about 14.4 million people and manages the screening activity in the south of England. Invitees are sent a guaiac-based faecal occult blood (gFOB) test kit, asked to provide a faecal sample and to return the test kit to the Hub for analysis. Participants with a positive ('abnormal') test are referred to a Specialist Screening Practitioner (SSP) for further investigation, usually colonoscopy.

Materials and methods: All screening activity, including uptake, gFOB test results, SSP referrals and colonoscopy outcomes are stored on the Bowel Cancer Screening System. Data for the period 2006-2011 were extracted and analysed.

Results: The uptake of screening invitations (the proportion of invitees that is adequately screened) is approximately 56% overall (higher for women than men [61% *vs*. 55%]). The proportion of positive test kits is higher for men than women (2.6% *vs*. 1.6%) and positivity has increased over time, with a consequent increase in the number of colonoscopies performed. About 40% of the screened population that undergoes colonoscopy has significant neoplasia (cancer, high- or intermediate-risk adenomas). The prevalence of significant neoplasia is greater in men and increases with age. The proportion of significant neoplasia detected in screening episode 2 is lower than in episode 1.

Conclusion: The screening data are encouraging and indicate that the BCSP in England is likely to achieve its goal of reducing bowel cancer mortality.

P13-07

Comparative study of the risk of developing NAFLD in individuals without liver disease

Almeida A, Pires C, Paulo R, Leitao C, Bellem F, Abreu R

Escola Superior Tecnologia Saude Lisboa, Laboratory Sciences and Technologies and Comunity Intervention, Lisbon, Portugal

Corresponding author: ana.almeida@estesl.ipl.pt

Introduction: Nonalcoholic fatty liver disease (NAFLD) has been recognized as the most common liver disease in Western countries, since its prevalence is high (20-30%) in developed countries. The objective of our work is a comparative study of biochemical parameters (glucose, cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides, AST and ALT) between individuals with NAFLD and others without NAFLD.

Materials and methods: Control group includes 47 subjects without NAFLD (25 females and 13 males) and other group with 11 subjects (2 females and 9 males) diagnosed with NAFLD by liver biopsy or ultrasound. After blood collection, the biochemical parameters were assessed on the equipment TARGA3000®.

Results: Significant differences were found between both groups on: triglycerides, AST and ALT. Aminotransferases are the variables that showed a more marked difference, AST and ALT values were elevated in 100% and 72,7%, respectively, of the subjects with NAFLD. One subject from control

group had higher values of aminotransferases then the average of subjects with NAFLD.

Conclusion: These results enlighten the need for surveying and monitoring apparently healthy population in order to be effective in primary prevention of NAFLD and other metabolic disorders.

P13-08

Citrulline – marker of enterocytes mass and function in patients after stem cells transplantation

Sedlackova T (1), Rajdl D (1), Ladislav T (1), Racek J (1), Vokurka S (2), Svoboda T (2)

(1) Charles University in Prague, Faculty of Medicine in Pilsen and Faculty Hospital in Pilsen, Department of Clinical Biochemistry and Haematology, Pilsen, Czech Republic
(2) Faculty Hospital in Pilsen, Department of Haemato-Oncology, Pilsen, Czech Republic

Corresponding author: terezka.sedlackova@seznam.cz

Background: Citrulline is an amino acid produced by enterocytes. Plasma citrulline concentration is considered as a marker of enterocytes mass and function. The determination of citrulline could be helpful in patients with intestinal failure, intestinal diseases (celiac and Crohn disease, etc.) or intestinal damage caused by toxicity of chemotherapy or graft-versus-host disease (GvHD).

Materials and methods: We measured plasma citrulline levels in 12 patients (20 patient´s samples) with diarrhea after allogeneic stem cell transplantation and in 20 healthy controls.

Results: The median value of citrulline levels was significantly lower in the transplanted patients group compared to healthy controls: 10.2 (0.6–63.8) *vs*. 33.3 (19.1–45.9) µmol/L, $P < 0.001$. The median values of citrulline levels in patients with post-transplant toxic intestinal damage (mucositis) (N = 8, day 1–22 post-transplant) *vs*. GvHD (N = 7, day 43-142) *vs*. "others" (usually dysmicrobia, N = 5, day 120-570) were: 9.6 (0.6–18.6) *vs*. 3.4 (1.9–9.9) *vs*. 19.5 (15.3–63.8) µmol/L.

Conclusions: The citrulline levels were significantly lower in patients compared to the control group. Low citrulline levels were found in patients shortly after stem cell transplantation and in patients with GvHD. Observations in larger groups of patients are necessary. Supported by specific fund SVV 262 806 and by the project Ministry of Health, Czech Republic for conceptual development of research organization 00669806 - Faculty Hospital in Pilsen, Czech Republic

P13-09

Prevalence of AMHA and ANA in patients with suspicious primary biliary cirrhosis

Padjen I (1), Kozmar A (2), Mayer M (1), Sentić M (1), Malenica B (2)

(1) University Hospital Centre Zagreb, Zagreb, Croatia, Department of Internal Medicine, Zagreb, Croatia
(2) University Hospital Centre Zagreb, Zagreb, Croatia, Department of Laboratory Diagnostics, Division of Immunology, Zagreb, Croatia

Corresponding author: akozmar@kbc-zagreb.hr

Primary biliary cirrhosis (PBC) is characterized by the presence of disease-specific autoantibodies that are primarily directed against mitochondrial antigens (AMA). However, a subgroup of patients' sera is also positive for antibodies to nuclear autoantigens (ANA). PBC-specific antinuclear antibodies are of diagnostic and clinical relevance since they can be used as a „positivie tool"in the diagnosis of AMA-negative PBC while at the same time identifying of patients with more advanced liver disease. Because both AMA and ANA testing are a critical part of diagnosis in PBC, it is important to detect them very carefully. Indirect immunofluorescences (IIF) on frozen sections of rat kidney and stomach, human epithelial HEp-2 cells and immunoblot to different target antigens have been used for this purpose. The aim of our study was to determine prevalence of PBC-specific ANA and their target antigens in 210 patients with suspicious PBC. AMA were positive in 91.9% (193/210) and PBC-specific ANA in 8.1% (17/210) of sera. Both AMA and ANA were found in 35.2% (74/210) of patients sera. AMA positive sera

recognized specific target autoantigens M2 and ANA specific autoantigens: Sp100 (57.1%; 52/91), PML (52.7%; 48/91) and gp210 (41.7; 38/91). Futhemore, they recognized the Ro-52 autoantigen (33.3%; 70/210). Sera that were ANA positive in the IIF recognized only nuclear specific autoantigens Sp100, PML and gp210. Our results show the compatibility of IIF and immunoblot in detecting AMA and ANA, and that IIF on HEp-2 cells and frozen sections of rat kidney and stomach detects PBC-specific ANA.

P13-10

Comparison of fecal calprotectin and CRP in pediatric inflammatory bowel disease

Linarić I (1), Obuljen J (1), Margetić S (2), Mišak Z (3), Hojsak I (3), Žižić V (1)

(1) Children's Hospital Zagreb, Department of Laboratory Diagnostics, Zagreb, Croatia
(2) Sestre milosrdnice University Hospital Center, Department of Laboratory Haematology and Coagulation, Universitiy Department of Chemistry, Zagreb, Croatia
(3) Children's Hospital Zagreb, Department of Gastroenterology and Nutrition, Zagreb, Croatia

Corresponding author: irenalinaric16@gmail.com

Background: The aim of the study was to evaluate the diagnostic accuracy of C-reactive protein (CRP) and fecal calprotectin (FC) as markers of inflammatory bowel disease (IBD) in pediatric patients.

Materials and methods: The study included 66 pediatrics patients; 41 patients with IBD (confirmed by colonoscopy as the gold standard) and 25 patients with excluded IBD (non-IBD). The serum concentrations of CRP were measured by an immunoturbidimetric high sensitive latex CRP assay (Beckman Coulter, AU 400 analyzer). The concentrations of FC were measured with commercially available enzyme-linked immunosorbent assay (Calprest, Eurospital).

Results: The medians (95% confidence interval (95%CI); interquartile range (IQR)) of both markers were significantly higher (P < 0.001) in IBD pediatric patients: CRP 8.7 mg/L (4.4-16.0; 2.7-20.6) and FC 368.0 µg/g (234.0-457.0; 215.8-559.0) compared with non-IBD patients (CRP 0.3 mg/L; (0.2-0.6; 0.2-0.7) and FC 15.6 µg/g (15.6-17.0; 15.6-20.8). However, the receiver operating characteristic (ROC) analysis showed significantly higher diagnostic accuracy (P = 0.039) of FC (area under curve (AUC) of 0.977; 95%CI = 0.905-0.998, sensitivity (Se) of 90.2%; 95%CI = 76.9-97.3, specificity (Sp) of 100%; 95%CI = 86.3-100.0, likelihood ratios LR- 0.09 and LR+ 22.6 at optimal cut-off value of 56 µg/g) compared with those of CRP (AUC = 0.903; 95%CI = 0.805-0.962, Se = 75.6% (95%CI = 59.7-87.6), Sp = 96.0% (95%CI = 79.6-99.9), LR- 0.25 and LR+ 18.9 at optimal cut off value of 2.6 mg/L).

Conclusion: Although both markers, CRP and fecal calprotectin, can be used for estimating mucosal inflammation in pediatric IBD patients, FC showed a higher diagnostic accuracy in discriminating between IBD and non-IBD pediatric patients with better sensitivity, specificity and likelihood ratios.

P13-11

Lipid profile in patients with chronic obstructive pulmonary disease

Milevoj Kopčinović L (1), Pancirov D (2), Simić L (3), Čepelak I (3), Žanić Grubišić T (3), Rumora L (3)

(1) Medical School University Hospital Sestre Milosrdnice, University Department of Chemistry, Clinical Unit of Medical Biochemistry in traumatology and orthopedics, Zagreb, Croatia
(2) Dr. Ivo Pedišić General Hospital, Department of Biochemistry and Hematology Diagnosis, Sisak, Croatia
(3) Faculty of Pharmacy and Biochemistry, University of Zagreb, Department of Medical Biochemistry and Hematology, Zagreb, Croatia

Corresponding author: laramilevoj@yahoo.com

Background: Chronic obstructive pulmonary disease (COPD) is a complex systemic disease associated with many comorbidities. Cardiovascular diseases (CVD) are the leading cause of death among patients with COPD. Our aim was to assess the lipid profile and the relationship between lipid and lung function parameters in COPD patients.

Materials and methods: The study included 38 healthy subjects (15 smokers, 10 ex-smokers, 13 non-smokers) and 103 COPD patients (31 smokers, 25 ex-smokers, 47 non-smokers). COPD patients were also subdivided according to disease severity (GOLD stages II-IV). FEV1 predicted and FEV1/FVC were determined by spirometry. Total cholesterol, triglycerides, HDL cholesterol, ApoA and ApoB were measured in sera of all participants, while LDL cholesterol and LDL/HDL and ApoB/ApoA ratios were calculated.

Results: Total cholesterol, triglycerides, LDL cholesterol and ApoA were lower, and ApoB/ApoA ratio was higher in patients with COPD ($P = 0.003$, $P < 0.001$, $P = 0.043$, $P < 0.001$, $P = 0.005$, respectively). No differences were found in HDL cholesterol and ApoB concentrations and LDL/HDL ratio when comparing patients with healthy subjects. However, HDL was weakly negatively correlated with FEV1/FVC in the patient group ($r = -0.27$, $P = 0.006$). In addition, neither disease severity nor smoking status influenced lipid parameters in COPD patients.

Conclusions: Although COPD is associated with an increased risk of CVD, our results do not confirm a pro-atherogenic lipid pattern in these patients. However, further research including a larger number of participants is needed to clarify this dilemma.

P13-12

MMP-9 and TIMP-1 concentrations in plasma of patients with chronic obstructive pulmonary disease

Somborac Bačura A (1), Rumora L (1), Šribar D (1), Popović-Grle S (2), Čepelak I (1), Žanić Grubišić T (1)

(1) University of Zagreb, Faculty of Pharmacy and Biochemistry, Department of Medical Biochemistry and Hematology, Zagreb, Croatia
(2) University Hospital Centre Zagreb, University of Zagreb, School of Medicine, Zagreb, Croatia

Corresponding author: asomborac@pharma.hr

Background: An imbalance of matrix metalloproteinases (MMPs) and tissue inhibitors of matrix metalloproteinases (TIMPs) has been implicated in the pathogenesis of chronic obstructive pulmonary disease (COPD). The aim of this study was to evaluate MMP-9 and TIMP-1 concentrations in COPD patients in relation to the severity of disease.

Materials and methods: The study included patients with stable COPD ($N = 59$) and healthy volunteers ($N = 21$). COPD patients were divided into subgroups (GOLD stages II to IV) according to the spirometry results. Plasma MMP-9 and TIMP-1 concentrations were determined using a commercially available ELISA kits. Classic inflammatory markers were also measured (differential leukocyte counts and CRP).

Results: MMP-9 concentration in COPD patients (204.13 (115.70-351.24) ng/mL) was significantly increased comparing to healthy controls (70.25 (52.48-104.96) ng/mL) with $P < 0.001$. There were no significant differences in TIMP-1 concentration. MMP-9/TIMP-1 ratio differed significantly between COPD patients (1.659 (0.965-2.687)) and healthy controls (0.627 (0.424-0.890)) with $P < 0.001$. Similar pattern was found already in GOLD II stage of disease. Very good diagnostic accuracy for MMP-9 was determined (AUC = 0.884; sensitivity of 66.1% and specificity of 100.0%; $P < 0.001$). The multivariate logistic regression model showed that the use of MMP-9 in combination with neutrophils, lymphocytes and CRP improved significantly ($P = 0.023$) diagnostic strength (AUC = 0.975).

Conclusions: Increased concentration of MPP-9 and higher MMP-9/TIMP-1 ratio found in COPD patients as early as in GOLD II stage highlight the significance of protease/antiprotease imbalance for the development of COPD and potential use of these parameters as biomarkers for early diagnosis of COPD.

P13-13

The relationship between BAL IL-8 and DLCO in patients with COPD

Fijačko M (1), Fijačko V (2), Dobrošević B (1), Pavela J (1), Sikora I (1), Šerić V (1)

(1) Clinical Hospital Center Osijek, Department of Clinical Laboratory Diagnostic, Osijek, Croatia
(2) Clinical Hospital Center Osijek, Department of Pulmonary Disease, Osijek, Croatia

Corresponding author: fijacko.mirjana@kbo.hr

Background: Most patients with chronic obstructive pulmonary disease (COPD) develop emphysema with alveolar destruction and small airway inflamation, most caused by smoking. The inflamation is characterised by increased neutrophils, CD8+ T lymphocyte, macrophages and associated cytokines, chemokines and proteases. Interleukin 8 (IL-8) is one of the best characterised members of the chemokine family and one of the most important neutrophil chemoattractants. Lung function measurements included FEV1, FEV1/FVC % and diffusing capacity (DLCO). Decrease DLCO results showed that gases do not diffuse normally across lung membranes, this indicate that certain lung disease are present: COPD or interstitial lung disease. The aim was to investigate relationship between DLCO and BAL IL-8.

Material and methods: 51 patients (82% man) with COPD and 16 controls (43% man) were studied. The concentrations of IL-8 was measured by ELISA method (eBioscience). DLCO was performed with single breath method on Master screen (Jaeger). Results of DLCO is usually reported as the percent of predicted amount of carbon monoxide inhaled that should be absorbed.

Results: Statistically significant differences were not found between BAL levels IL-8 in COPD patients and control group (504 ± 666 pg/mL *vs*. 264 ± 256 pg/mL). Statistically significant differences were found between DLCO in COPD patients and control group (66.4 ± 22.8 % *vs*. 4.4 ± 23.0 at P level < 0.05). In addition, BAL IL-8 in COPD patients showed a strong negative correlation to DLCO (r= - 0.40, P < 0.05.).

Conclusions: These results showed that BAL IL-8 was signifficantly associated with DLCO in patients with COPD.

P14 - Microbiology - Infection

P14-01

Screening for the urinary tract infections using Sysmex UF 1000i flow cytometer

Konderak J (1), Elmardi A (2), Farkas B (2), Pinter E (1)

(1) Synlab Hungary Ltd., Medical Biochemical Department, Budapest, Hungary
(2) Synlab Hungary Ltd., Microbiological Department, Budapest, Hungary

Corresponding author: jkonderak@gmail.com

Background: Bacterial cultures for the urinary tract infections (UTI) are the most common microbiological tests. Huge amounts of negative cultures demand for effective screening method reducing cost and time. The aim of this study was to evaluate the efficacy of UF 1000i flow cytometer for preselecting of negative results.

Material and methods: Urine specimens (N = 1226) suspected UTI were simultaneously cultured and analyzed by UF 1000i for bacterial (BC) and leukocyte (WBC) counts. Population: male:female = 1:2; ages: 0-15 years: 8%; 16-65 years: 59%; > 65 years: 33%; outpatient:inpatient = 4:1. For culture samples were inoculated using a 10 µL loop on selective agar plates. After standard incubation results were evaluated. A sample was considered negative for UTI if growth was < 103 CFU/mL (colony forming unit). Positive samples were attributed to one of the levels of CFU/mL (> 103, > 104, > 105).

Results: Using culture results as gold standard, we performed ROC analysis to determine area under curve (AUC), cutoff values (CO), sensitivities (SE), specificities (SP), negative predictive values (NPV) in pointing to BC and WBC measured by UF1000i.

Results of ROC statistics at CFU/ml of > 103, > 104, > 105 AUC: 0.88, 0.91 and 0.93, CO BC and/or WBC/µL: > 9, > 22, > 100, SE%: 95, 96, 96; SP%: 31, 45, 54; NPV%: 97, 98, 98, respectively.

Conclusions: By screening out method, UF 1000i saved 23, 32 and 42% of the total urine cultures at > 103, > 104 and > 105 CFU/mL respectively.

P14-02

Comparison between a syphilis screen of two immunoassays for the diagnosis of syphilis

Saue E, Viia V, Mägi M

East-Tallinn Central Hospital Diagnostic Clinic, Central Laboratory, Tallinn, Estonia

Corresponding author: elo.saue@itk.ee

Background: Syphilis can be asymptomatic, serologic screening is recommended for persons at high risk, pregnant women, blood donors and routine survey. *Treponema pallidum*, the bacterium that causes syphilis, cannot be cultured. Serologic testing is treponemal method most often used to diagnose syphilis in patients with suspected disease. To evaluate the performance of the Immulite 2000 Syphilis Screen assay to the Cobas 6000 Treponema pallidum latex agglutination assay-TP-LA. Parallel studies were carried out in order to switch to another method of analysis of the syphilis screening.

Materials and methods: Out of total 43 human serum specimens, belonging to three different categories (routine laboratory screening for syphilis, N = 16; syphilis patients, N = 15; potential false-positive results, N = 12), parallel studies were carried out in 16 unselected and 27 previously maintained positive serums. During the analysed period, parallel studies were performed by using the chemiluminescent immunoassay (Immulite 2000) and latex agglutination assay (Cobas 6000) method. All samples were tested with immunoblot Treponema IgG and IgM kits for confirmation.

Results: 6 results differed from the total number of 43 parallel studies carried out. 6 results were found to be false-positive since the confirmatory tests affirmed negative results. Syphilis screening can be switched over to the Cobas 6000 analyzer.

Conclusions: In comparison with the Syphilis Screen assay, the TPLA assay is more specific than the Immulite kit and has the advantage of reducing the number of confirmatory tests (mostly pregnant sera).

P14-03

Procalcitonin as early diagnostic marker for infection in febril neutropenia cancer patients

Alonso T (1), Cuadrado-Cenzual M (2), De Gracia Y (2), Ortega De Heredia D (2), Perez-Segura P (1)

(1) Hospital Clinico San Carlos, Oncologia, Madrid, Spain
(2) Hospital Clinico San Carlos, Analisis Clinicos, Madrid, Spain

Corresponding author: mcuadrado.hcsc@salud.madrid.org

Introduction: Febrile neutropenia (FN) in cancer patients is a complication related to antineoplasic therapy with serious impact on their morbidity and survival. The main cause is the infectious process. The aim of this study was to evaluate Procalcitonin (PCT) like early diagnostic marker in patient with FN associated with chemotherapy.

Materials and methods: A prospective double blind study was developed. We included 78 patients with FN and with treatment to them of the fever according to clinical practice protocol. Of all of them, 61 patients completed all the study with serial studies of PCT serum levels.

Results: Bateriemia was detected in 36 patients and PCT showed higher significantly levels (P < 0.001) in this patients in comparision with non bacteriemia patients. Relation between Procalcitonine values and treatment non–response was significant (p 0.999). The point of cut-off PCT level, with better sensitivity (74.6 %) and specificity (80%) was 0.21µg/L. The multivariant analysis

showed that the value of PCT over 0.5 µg/L in patients with FN and bacteriemia it was an independent variable like marking diagnosis of bacteriemia in patients with febrile neutropenia (Odss Ratio 3.5 with an interval of 95% confidence 1.6-7.8) and P < 0.001.

Conclusion: PCT values in cancer patients with FN and infections; was higher and descend after restoring antibiotic treatment agreeing with clinical improvement. These data suggest it determination of PCT in cancer patients with FN; could be an useful early diagnostic marker for detection of bacteriemia

P14-04

The role of procalcitonin in sepsis patients with pneumonia

Cachapuz I (1), Amaro A (2), Granja C (2), Alves V (1)

(1) Matosinhos Local Health Care Unit, Clinical Pathology Service, Oporto, Portugal
(2) Matosinhos Local Health Care Unit, Intensive Care Medicine Service, Oporto, Portugal

Corresponding author: cachapuz.isabel@gmail.com

Background: The value of procalcitonin (PCT) in acutely ill sepsis patients has been studied and the use of this biomarker revealed its clinical relevance in ICU setting. The study purpose was to evaluate the role of PCT on septic patients management with community-acquired pneumonia (CAP) and invasive ventilator-associated pneumonia (IVAP).

Materials and methods: Prospective study. Adults admitted in ICU (April - December 2010) with CAP and/or IVAP diagnosis. PCT and C-reactive protein (CRP) were dosed daily. All patients underwent microbiological testing. Severity indexes were assessed. SPSS(v16) was used for statistical analysis.

Results: Total of 43 patients: 37 with sepsis related to CAP; 6 with sepsis related to IVAP. 74%male, average age:57 years old. 70% of the cases had a septic shock and 21% had severe sepsis. Blood cultures were positive in 11.6%; 25.6% had positive tracheal aspirate cultures; 26% were positive for antigenuria. Mean SOFA: 9 (SD 3.9);APACHE II: 20 (SD 8.5). Length of stay: 11.1 days. Mortality:14.7%. On admission day, PCT mean value: 23.7 ng/mL and CRP: 20.9 mg/dL. On day 1 serum PCT revealed a significant correlation with APACHE II index (P < 0.05) and was positive for blood cultures (P < 0.05).But, CRP value did not reveal these correlations. There was no relation either between PCT and SOFA index or with mortality.

Conclusions: This study revealed that even though PCT is related to APACHE II severity index, it is not related to ICU mortality. We found a significant correlation between PCT value and positive blood cultures, as mentioned in previous publications.

P14-05

Extended spectrum Beta-lactamases and AmpC Beta lactamases producing uropathogenes among children

Deo P

Monash University, Department of Biochemistry and Molecular Biology, Melbourne, Australia

Corresponding author: pdeo2@student.monash.edu

Objectives: UTI are common in childhood. Gram negative bacilli are the common isolates from the UTI. ESBL and AmpC beta-lactamase are the most significant enzymes involved in conferring resistance to Beta-lactam antibiotics in Gram negative bacteria. This study was aimed to find out the prevalence of multidrug resistant (MDR), ESBL and AmpC beta-lactamases producing isolates among children with UTI in Nepal.

Materials and methods: A prospective study was carried out from July, 2006 to March, 2008 at IOM,Kathmandu, Nepal). 820 urine specimens were obtained from clinically suspected UTI children (age < 12 years, female to male ratio 2.1:1). Most of samples were midstream urine, 25 supra pubic aspiration and 4 from catheter. Culture, organism identification and antibiotic susceptibility

test were done by following the protocol of American Society for Microbiology (ASM).

Results: Among 820 urine samples, 23.51% (201/820) had significant bacterial growth with 184 (91.54%) non-repeat gram-negative isolates in which most were *E. coli* (58.15%) followed by *Klebsiella* species (15.2%).The prevalence of MDR, ESBL and AmpC were 115 (62.5%), 43 (23.36%) and 15 (8.15%) respectively. Maximum incidence of ESBL producer was *E. coli* (39.5%) followed by *Klebsiella* (16.2%) and *Pseudomonas* species (13.9%). Highly AmpC producing species were *Klebsiella* (40%) and *Pseudomonas* (26.6%). ESBL producers and non producers MDR isolates were highly resistant to amoxycillin-clavunic acid, aztrenome, cefepime and ceftazidime-clavunic acid. Imepenum and pipracilin were most effective drug among ESBL producers and non producers. Infection was more common in age group 6 ± 2.3 years with female to male ratio 2.04:1.

Conclusion: Result shows high percentage (62.5%) of MDR pathogens in childhood UTI.

P14-06

Mass spectrometry in identification of antibiotic resistance

Pilatova K (1), Frostova T (1), Dolejska M (2), Budinska E (3), Sterba J (4), Zdrazilova Dubska L (1)

(1) Masaryk Memorial Cancer Institute, Department of Laboratory Medicine, Brno, Czech Republic
(2) University of Veterinary and Pharmaceutical Sciences, Department of Biology and Wildlife Diseases, Brno, Czech Republic
(3) Masaryk University, Institute of Biostatistics and Analyses, Brno, Czech Republic
(4) Clinic of Pediatric Oncology, Children's Medical Center, Brno, Czech Republic

Corresponding author: katerina.pilatova@mou.cz

Background: The assessment of antibiotic resistance is essential for appropriate treatment initiation of infection disease. Increasing frequency of multiresistant bacterial strains and development of new antibiotic resistance mechanisms represent major problems. Currently, mass spectrometry (MS) represents new approach for bacterial identification. The goal of this study is assessment of correlation between MS profile and bacterial antibiotic fenotype.

Materials and methods: We analyzed 56 isolates of Enterobacteriacea obtained from children treated for malignant disease. Bacteria were cultivated on agar plates; antibiotic susceptibility was tested using disc diffusion method, ESBL strains by double-disc synergy test. Sample preparation for SELDI-TOF MS: one bacterial colony was resuspended in 100 µL of distilled water and frozen at -70 °C. After thawing, suspension was applied on protein gold chip and after drying, covered twice by sinapinic acid matrix. Measurement was carried out on SELDI-TOF MS (Ciphergen) in the range of m/z = 3000 - 20000.

Results: We were able to clearly distinguish between individual bacterial species on the basis of cluster analysis of acquired protein spectra. We also found peaks which differ significantly in individual strains and correlates with antibiotic fenotype in Klebsiella pneumoniae.

Conclusions: Our pilot data show that protein profiling of bacteria using mass spectrometric methods allows discovery of new antibiotic resistance biomarkers and might represent new approach for identification of resistant bacteria strains as alternative method for current cultivation and molecular biology methods. This study was supported by European Regional Development Fund (RECAMO; CZ.1.05/2.1.00/03.0101) and by the Czech Science Foundation (grant P502/10/P083).

P14-07

Prevalence of syphilis among blood donors in years 2009-2011 and our experiences with Abbott and Siemens reagents

Sopić T

University Clinical Centre of Maribor, Centre of Transfusion Medicine, Maribor, Slovenia

Corresponding author: tanja.sopic@ukc-mb.si

Background: Syphilis is sexually transmitted disease, caused by spirochaete organism *Treponema pallidum*. Although rare, it can be transmitted with blood transfusion and also is a good indicator of high risk sexual behaviours. Testing blood donors for syphilis is mandatory in most countries of the world.

Materials and methods: Specimens of blood donors were tested with Abbott reagents on Architect with CMIA (Chemiluminiscent Immunoassay) method and with Siemens reagents on BEP 2000 with EIA (Enzyme Immunoassay) method. Initially reactive samples were tested in duplicate. Repeatedly reactive samples were sent to confirmatory testing in reference laboratory.

Results: In years 2009-2011 we have tested 94,328 blood donations, 42,543 with Abbott reagents and 51,785 with Siemens reagents. There were 97 (0,23 %) initially reactive and then 93 (0.22%) repeatedly reactive with Abbbott reagents and 62 (0.12%) initially reactive and then 43 (0.08) repeatedly reactive with Siemens reagents. Confirmatory testing was positive for 17 (0.02 %) samples.

Conclusion: All confirmed positive results were reactive with Abbott and Siemens reagents, so both are suitable for screening for syphilis. We noticed more more false reactive results with Abbott reagents, then with Siemens reagents. The prevalence of syphilis among blood donors is staying the same in the last three years.

P14-08

HBV pre-existing immunity of healthcare workers in occupational exposures in University Hospital Dubrava

Serdar T (1), Markovic D (2), Marijancevic D (1), Petrovecki M (3), Romic Z (1)

(1) University Hospital Dubrava, Clinical Department for Laboratory Diagnostic, Zagreb, Croatia
(2) University Hospital Dubrava, Department for Clinical Microbiology and Hospital Infections, Zagreb, Croatia
(3) Clinical Department for Laboratory Diagnostics, University Hospital Dubrava, Zagreb, Department of Medical Informatics, Rijeka University School of Medicine, Rijeka, Croatia

Corresponding author: tserdar@kbd.hr

Background: Occupational exposure in healthcare workers represents every contact with a material that carries the risk of acquiring an infection during the working activities. Viral infections are the main blood transmitted infections and the most frequent are hepatitis B virus (HBV), hepatitis C virus (HCV) and human immunodeficiency virus (HIV).

Materials and methods: Since 2002, every reported occupational exposure of healthcare worker was registrated by the Committee for hospital infections of University Hospital Dubrava. Database among others contains qualifications of the exposed worker and his pre-existing immunity to HBV (aHBs-titer).

Results: In 10 year period of occupational exposures monitoring (from 2002 to 2011) 451 cases were reported. The majority of occupational exposures were reported by nurses or medical technicians (55.4%), followed by medical doctor resident (12.6%), medical doctor specialist (8.0%), cleaner (6.9%), laboratory technician (3.3%) and others (13.8%). In 59.4% of the exposed healthcare workers aHBs-titer status was assessed as satisfactory (aHBs > 100 IU/L), in 19.5% was not satisfactory (aHBs < 100 IU/L) and in 21.1% was not measured.

Conclusion: The 10-year follow-up of occupational exposures in healthcare workers in University Hospital Dubrava has shown an increase in the

number of reported events. Implementation of preventive measures, such as universal precaution measures and HBV vaccination results a significant reduction in the incidence of HBV infection among healthcare workers. In many countries, the number of healthcare workers that underwent HBV vaccination usually does not exceed 65%, indicating that HBV vaccination is not applied in a sufficient number of healthcare workers.

P14-09

Trends in antibiotics prescribed for bacterial pneumonia with higher CRP in preschool children

Bajraktarevic A (1), Kaljic Junuzovic A (1), Miokovic M (1), Penava S (1), Mulabegovic J (2), Delic M (3)

(1) Public Health Institution of Canton Sarajevo, Pediatrics department, Sarajevo, Bosnia and Herzegovina
(2) Pharmacy Faculty, Department for Biochemistry and Clinical Pharmacologz, Sarajevo, Bosnia and Herzegovina
(3) Krankenhaus Hietzing Stadt Wien Mit Neurologishem Zentrum Rosenhögel, Insitute Für Labormedizin Mit Serologie Und Infektiondiagnostik Mit Ambulanz , Vienna, Austria

Corresponding author: bajrakm@seznam.cz

Objective: To estimate the incidence rates of preschool children outpatient bacterial pneumonia, examine time trends in antibiotics prescribed for bacterial pneumonia with higher CRP, and determine factors associated with broad-spectrum antibiotic prescribing for pneumonia in this population.

Materials and methods: The material consists of 902 small preschool Bosnians children aged 6 months to 6 years or half year more who took part in this retrospective study in pediatrics settings in six municipalities from nine municipalities of Canton Sarajevo. Within 72 or 96 hours after establition of diagnosis and beginning of antibiotics therapy blood samples CRP were taken for first and control analysis.

Results: The four most commonly prescribed antibiotic classes for bacterial pneumonia in preschool children were cephalosporins, macrolides, penicillins and aminoglycoside antibiotics. Cephalosporins were most commonly prescribed, ranging from 33.3% to 44.5% of all antibiotics prescribed for pneumonia in ages from 6 months to 6 years. Macrolides were the second most commonly prescribed antibiotic, ranging from 22.5% to 35.3% of all antibiotics prescribed for this diseases. There was no statistical difference in serum CRP values among the four groups with pneumonia after antibiotics therapy.

Conclusions: A strong association has been found between the level of circulating C-reactive protein (CRP) and the severity of pneumonia and success of antibiotics therapy in control laboratory data after three or four days in outpatients conditions .

P15 - Molecular diagnosis 1

P15-01

The association of postprandial triglycerides with hsCRP, TAS, ICAM-1 and APOA5 and HL gene variants

Kackov S (1), Simundic AM (2), Nikolac N (2), Celap I (2), Dukic L (2), Bilusic M (1)

(1) Policlinic Bonifarm, Medical biochemistry laboratory, Zagreb, Croatia
(2) Sestre milosrdnice University Hospital Center, University Department of Chemistry, Zagreb, Croatia

Corresponding author: sanjakackov@hotmail.com

Background: Several authors have reported the association of postprandial hypertriglyceridemia with oxidative stress, systemic inflammation and endothelial dysfunction. Our aim was to investigate the effect of postprandial hypertriglyceridemia on oxidative stress and endothelial dysfunction. We also assessed the association of APOA5 -1131T/C and -250G/A hepatic lipase polymorphisms with different response to the high-calorie meal in the group of healthy middle aged male individuals.

Materials and methods: We recruited 102 healthy male volunteers (52-68 years). All participants con-

sumed a high-calorie meal (800 calories, 50 g fat, 28 g protein, 60 g carbohydrates). Blood samples were drawn at 8 a.m., after an overnight fast and 3 hours after the meal. Glucose, total cholesterol, triglycerides, HDL-cholesterol, LDL-cholesterol, bilirubin, uric acid, hsCRP, TAS and ICAM-1 were measured at fasting state and postprandially. APOA5 -1131T/C and -250G/A hepatic lipase promoter genetic polymorphisms were determined for all participants.

Results: Postprandial triglycerides were significantly increased (1.4 (1.1 - 2.1) *vs*. 2.4 (1.9 - 3.3) mmol/L, $P < 0.001$). Average triglyceride increase was 1.0 ± 0.7 mmol/L (65%). Although concentrations of triglycerides, HDL-cholesterol, LDL-cholesterol, TAS and ICAM-1 differed significantly between fasting state and postprandial measurements ($P < 0.001$), differences were within the limits of analytical imprecision and are not considered as clinically relevant. Other parameters did not change 3 hours after the meal. Triglycerides response did not differ respective to the APOA5 and HL polymorphisms.

Conclusion: Postprandial hypertriglyceridemia is not associated with increased concentrations of hsCRP, TAS and ICAM-1. Furthermore, APOA5 and HL polymorphisms are not associated with different response of triglycerides.

P15-02

Association of three polymorphisms of scavenger receptor class BI gene in with coronary stenosis

Bouslama A

Sahloul University Hospital, Biochemistry, Sousse, Tunisia

Corresponding author: ali.bouslama@rns.tn

Background: The potential role of scavenger receptor class BI in the regulation of lipoproteins metabolism and atherosclerosis has attracted considerable interest. We tested the relationship of three *SCARB1* polymorphisms with significant coronary stenosis (SCS) and lipid profile in a coronary Tunisian population.

Materials and methods: Three *SCARB1* polymorphisms (exon8 (C/T), exon1 (G/A), intron5 (C/T)) were studied in 316 tunisians undergoing coronary angiography. SCS was defined as a luminal narrowing of ≥ 50% in at least one major coronary artery. Lipid profile was measured. Genotyping was performed using PCR-RFLP. Statistical analysis was performed by SPSS.

Results and conclusion: TT genotype of exon8 was associated with higher concentrations of HDL-C and ApoAI in the group without SCS. The T allele of exon 8 was associated with 41% lower risk of SCS. This protective effect seemed to be particularly significant in women, non diabetics and nonsmokers. The T allele of intron 5 was associated with an increased risk of SCS, particularly in smokers. AA genotype of exon1 was associated with an increased risk of SCS in diabetics and in patients with metabolic syndrome. The (CAT) haplotype was associated with an increased risk of SCS compared to the wild haplotype and had a 4-fold greater risk of SCS than patients with haplotype (TGC) which seems to be the most protective against SCS. The T allele of exon 8 in SCARB1 seemed to increase the HDL-C and ApoAI concentrations and reduce the risk of SCS. The intron 5, exon 1 polymorphisms and (CAT) haplotype seemed to have an atherogenic effect.

P15-03

Six lipoprotein lipase gene polymorphisms, lipids and coronary stenosis in a Tunisian population

Rebhi L (1), Kchok K (1), Omezzine A (1), Rejeb J (1), Boughzala E (2), Bouslama A (1)

(1) Sahloul University Hospital, Biochemistry, Sousse, Tunisia
(2) Sahloul University Hospital, Cardiology, Sousse, Tunisia

Corresponding author: ali.bouslama@rns.tn

Background: Lipoprotein lipase (LPL) is the rate-limiting enzyme in the hydrolysis of triglyceride-rich lipoprotein particles. LPL polymorphisms' effects on lipids and coronary artery disease are controversial among studies and populations. Our aim was to study the association between six polymorphisms and significant coronary stenosis (SCS), disease severity and lipid parameters in Tunisian patients.

Materials and methods: LPL *PvuII*, 93 T/G, 188 G/E, *HindIII,* N291S and D9N polymorphisms were analyzed in 316 patients who underwent coronary angiography. SCS was defined as the presence of stenosis ≥ 50% in at least one major coronary artery. The stenosis severity was determined by using Gensini score (GS).

Results and conclusions: A significant association of SCS with TT and TG genotypes of the *HindIII* polymorphism was showed: OR = 2.84, 95% CI, 1.19-7.40, P = 0.017; OR = 1.77, 95% CI, 1.99-2.82, P = 0.033), respectively. The TT genotype was significantly associated with increased triglyceride level and ApoB/ApoA-I ratio and with decreased HDL-C. Haplotype analysis showed that OR of SCS associated with the CTGTAG haplotype was 2.12 (95% CI 1.05-4.25,P = 0.032) and with CGGGAA was 0.71 (95% CI 0.26-1.95, P = 0.022) compared to the CTGTAA. Significant difference in GS was observed among *HindIII* genotypes and haplotypes. A significant association between the mutated genotype of *HindIII* polymorphism with decreased HDL-C level and increased ApoB/ApoA-I ratio and triglyceride level was showed. Our results suggest that *HindIII* and D9N polymorphisms and CTGTAG haplotype seem to be considered as marker of coronary stenosis. In another hand, *HindIII* and haplotypes were related to coronary stenosis severity.

P15-04

Toll-like receptors tlr-2 and tlr-4 gene polymorphisms in patients with cerebral atherosclerosis

Galovic R (1), Flegar-Mestric Z (2), Matokanovic M (3), Brajsa K (4), Vidjak V (5), Barisic K (3)

(1) University Hospital Centre Zagreb, Clinical Dept. of Laboratory Diagnostics, Zagreb, Croatia
(2) University Hospital Merkur, Institute of Clinical Chemistry and Laboratory Medicine, ZAGREB, Croatia
(3) University of Zagreb Faculty of Pharmacy and Biochemistry, Dept. of Medical Biochemistry and Hematology, Zagreb, Croatia
(4) Galapagos Research Center Ltd., Research Center, Zagreb, Croatia
(5) University Hospital Merkur, Institute for Radiology, Zagreb, Croatia

Corresponding author: zlata.mestric@gmail.com

Background: The innate immunity proteins TLR-2 and TLR-4 are possible connection between immune response and inflammation involved in the pathogenesis of atherosclerosis. Previous studies suggest that tlr-2 and tlr-4 gene polymorphisms could contribute to the differences in the disease development.

Materials and methods: A group of patients with cerebral atherosclerosis, evaluated by digital subtraction angiography, with > 50% stenosis of cerebral artery (N = 47) was compared to the control group (N = 27) concerning single nucleotide polymorphisms G/A753 in the tlr-2 gene and A/G299 in the tlr-4 gene, determined by the real-time PCR. Serum proinflammatory cytokines IL-6 and TNF-α, were determined by enzyme immunoassays.

Results: Examined polymorphism of tlr-2 gene was present in 3.7% and 5.3% subjects of the control and of the cerebral atherosclerosis group, respectively, while polymorphism of tlr-4 in 9.3% and 8.5% subjects, respectively. There was no difference in

proportions of the polymorphisms between the groups. The concentrations of IL-6 and TNF-α were higher in the cerebral atherosclerosis than in the control group (1.54 pg/mL, (1.04-3.50 pg/mL) *vs*. 1.21 pg/mL, (0.45-2.32 pg/mL), P = 0.048 and (0 pg/mL (0-1.8 pg/mL) *vs*. 0 pg/mL, (0-0 pg/mL), P = 0.039, respectively), presented as median (1-3 quartile).

Conclusion: Higher concentrations of IL-6 and TNF-α in the cerebral atherosclerosis group are indicators of inherent inflammation. Single nucleotide polymorphisms G/A573 of the tlr-2 and A/G299 of the tlr-4 gene had no influence on the concentrations of circulating IL-6 and TNF-α. The polymorphisms were not significant for the diagnosis of cerebral atherosclerosis with more than 50% stenosis of cerebral arteries.

P15-05

Four resistin polymorphisms, metabolic syndrome parameters and obesity risk in Tunisian volunteers

Boumaiza I, Omezzine A, Rejeb J, Rebhi L, Ben Rejeb N, Bouslama A

Sahloul University Hospital, Biochemistry, Sousse, Tunisia

Corresponding author: ali.bouslama@rns.tn

Background: Resistin is a protein hormone produced by adipocytes. Some studies found increased circulating resistin levels and its mRNA expression in adipose tissue in patients with obesity. While other studies failed to confirm this finding. Genes encoding adipokines are important functional candidates for development of obesity. Many resistin gene polymorphisms were described and their implication in obesity and metabolic syndrome (MetS) was controversial. Our aim was to study the relationship between four resistin polymorphisms (420C/G, 44G/A, 62G/A and 394C/G) and MetS parameters and the risk of obesity in Tunisian volunteers.

Materials and methods: We have recruited 169 non obese BMI < 30 kg/m^2 (sex-ratio = 0.594, mean age 43.25 ± 13.12 years; mean BMI 24.73 ± 3.50 kg/m^2) and 160 obese BMI ≥ 30 kg/m^2 (sex-ratio = 0.221, mean age 48.41 ± 10.92 years, mean BMI 36.6 ± 4.8 kg/m^2). Genotyping was performed using PCR-RFLP. Lipids parameters were measured. BMI and HOMA-IR were calculated. MetS was defined according to IDF-2005, obesity was defined according to WHO-1995. The study was approved by the Medical Hospital Ethic Committee.

Results and conclusion: 420GG were associated with higher waist circumference and BMI. 44G/A polymorphism was associated with increased total cholesterol and LDL-C levels. The others genotypes showed no association with all MetS parameters. Concerning association between SNPs and MetS risk, only mutated genotypes at 44G/A increase the risk of MetS after adjustment to confounders parameters (OR = 1.93, P = 0.023). About obesity risk, only 420C/G seems to contribute in obesity. Adjusted ORs of obesity associated to mutated genotypes were 2.17, 95%CI [1.28-3.68]; P = 0.004.

P15-06

Simultaneous detection of mutations within genes associated with familial hypercholesterolemia

Latten M (1), Murray H (1), Graham C (2), Martin R (2), Crockard M (1), Fitzgerald S (1)

(1) Randox Laboratories Limited, Molecular Diagnostics, Crumlin, United Kingdom
(2) Northern Ireland Regional Genetics Centre, Belfast Health and Social Care Trust/ City Hospital, Belfast, United Kingdom

Corresponding author: Julie-Ann.Mayne@randox.com

Background: Familial Hypercholesterolemia (FH) is a genetic disorder characterised by high levels of low density lipoprotein in the cardiovascular system and early onset of cardiovascular disease. Currently in the UK, 1 in 500 people suffer from FH, with 85% of this population un-diagnosed. The genes apo-lipoprotein B, low density lipoprotein receptor, and proprotein convertase subtilisin/kexin type 9 are known to be associated with FH. This study reports the development of an assay enabling the simultaneous analysis of 20 common mutations within these genes.

Materials and methods: The assay is based on a combination of two multiplex PCRs and biochip array hybridisation. Innovative PCR priming technology permits high discrimination between multiple wild-type and mutant DNA regions, which in combination with spatially organised biochip array technology increases the multiplexing capacity of the assay. Dedicated software processes results automatically, with analysis completed within 3 hours, from template DNA.

Results: Assay specificity was confirmed using DNA from 100 FH positive patient samples. Subsequently, a further 100 blinded samples were assessed with a correlation of 98% with patient samples that had previously been sequenced. The cohort included patient samples containing the 20 common mutations.

Conclusions: Data indicates applicability of this assay for the rapid simultaneous analysis of 20 common mutations within three genes associated with FH. Treatment from adolescence age with lipid modifying drug therapy, combined with lifestyle changes, can restore normal life expectancy. Therefore, this assay can be used as an analytical tool to facilitate FH diagnosis.

P15-07

Apolipoprotein A5 genetic polymorphisms and fasting serum lipidogram in elderly subjects with MetS

Feher Turkovic L (1), Pasalic D (2), Pavlovic M (3)

(1) University of Applied Health Studies-Zagreb, Department of Chemistry, Biochemistry and Clinical Chemistry, Zagreb, Croatia
(2) University of Zagreb, School of Medicine, Department of Medical Chemistry, Biochemistry, and Clinical Chemistry, Zagreb, Croatia
(3) Institute for Medical Research and Occupational Health, Department of Occupational Health and Environmental Medicine, Zagreb, Croatia

Corresponding author: daria.pasalic@mef.hr

Background: Metabolic syndrome (MetS) is a cluster of metabolic abnormalities as are obesity, dyslipidemia, hyperglycemia, and hypertension. It represents a risk factor for many disorders. Apolipoprotein A5 which is located on VLDL and HDL regulates the concentration of triglycerides. Therefore, genetic isoforms might affects triglyceride concentration and contribute in pathogenesis of MetS.

Materials and methods: The cross-sectional study included 155 men and 187 women older than 70 years. Fasting serum concentration of biochemical parameters were determined by standardized methods. International Diabetes Federation criteria was used for determination of MetS. Two *Apo A5* genetics polymorphisms (c.1259T>C-SNP1 andS19W) were genotyped using PCR-RFLP method with detection of fragments with chips on Agilent 2100 bioanalyzer.

Results: SNP1 genotype frequencies for T/T, T/C and C/C genotype were 183, 41 and 1 in MetS(+), and 96, 15 and 11 in MetS(-), respectively, (P = 0.478). S19W genotype frequencies for SS, SW and WW were 184, 41 and 2 in MetS(+) and 92, 22 and O in MetS(-), (P = 0.586). Mean total-cholesterol concentration was significantly higher in SNP1-C allele carriers then in non-carriers for women in MetS(+) group (6.23 ± 1.36 and 5.58 ±1.14, respectively, P = 0.020). LDL-cholesterol was also significantly higher for same group and same allele (4.05 ± 1.04 and 3.53 ± 0.97, P = 0.026). In subjects with MetS(+), higher triglycerides concentration were observed in W-carriers than in non-carriers of S19W (median were 1.79 and 1.97, respectively, P = 0.050). Total-cholesterol were higher in men W-carriers *vs*. non-carriers (5.55 ± 1.17 and 4.97 ± 1.11 respectively, P = 0.048).

Conclusions: Apo A5 genetic polymorphisms SNP1 and S19W are associated with dislypidemia in elderly subjects with MetS.

P15-08

Adiponectin gene variants, and gene-environment interactions as predictors of early central obesity

Karmelić I (1), Lovrić J (1), Sertić J (2), Božina T (1), Božina N (2), Jelaković B (3)

(1) School of Medicine, Department of Chemistry, Biochemistry and Clinical Chemistry, Zagreb, Croatia
(2) University Hospital Centre Zagreb, Department of Laboratory Diagnostics, Zagreb, Croatia
(3) University Hospital Centre Zagreb, Department of Internal Medicine, Division of Nephrology and Arterial Hypertension, Zagreb, Croatia

Corresponding author: k.ivanakarmelic@gmail.com

Background: Adiponectin is an adipose tissue-derived adipokine linked to central obesity and *ADIPOQ* variants are promising markers for understanding the genetic base of obesity. We aimed to evaluate the relation of adiponectin concentrations and *ADIPOQ* gene variants to abdominal obesity and hypertension in young subjects. In addition, influence of the gene-environment (diet) interaction was estimated.

Materials and methods: The study included 149 subjects. Clinical examination and anthropometric measurements were done. Adiponectin levels were estimated by ELISA assay. *ADIPOQ* -11377C>G and -11391G>A were genotyped by real-time PCR.

Results: Waist circumference, systolic and diastolic blood pressure showed inverse correlations with adiponectin concentrations. *ADIPOQ* -11377GG and -11391GA significantly increased the risk for the development of central obesity (OR 5.57 and OR 3.37, respectively). The test of overall association showed significant correlation of central obesity with -11377C>G and -11391G>A haplotypes ($P < 0.001$). We found a significant association of -11391G/A variants with triglycerides and BMI, with A allele more frequent in subjects with BMI ≥ 25 kg/m^2 ($P = 0.021$), while GG genotype predispose for lower concentrations of triglycerides ($P = 0.005$). A significant correlation was found for -11377GG variant with the concentration of total cholesterol (G/G *vs*. other $P = 0.043$) and hypertension ($P = 0.035$), where -11377G allele carriers have significantly higher risk for elevated blood pressure (OR 2.73). When a diet was introduced as a covariable, correlation was significant between -11391G>A and HDL-C only ($P = 0.015$).

Conclusion: Analysis of adiponectin concentration and *ADIPOQ* -11391G>A and -11377C>G promoter gene variants could be clinically meaningful for estimation of obesity and obesity-related syndrome risk in young adult population.

P15-09

Gender-specific effects of PPARG, APOE, ACE, LPL, IL-6 and AT1R gene variants on metabolic syndrome

Božina T (1), Sertić J (2), Lovrić J (1), Jelaković B (3), Merkler M (4), Reiner Ž (4)

(1) School of Medicine, University of Zagreb, Department of Medical Chemistry, Biochemistry and Clinical Chemistry, Zagreb, Croatia
(2) University Hospital Centre Zagreb, School of Medicine, University of Zagreb, Department of Laboratory Diagnostics, Department of Medical Chemistry, Biochemistry and Clinical Chemistry, Zagreb, Croatia
(3) University Hospital Centre Zagreb, Department of Internal Medicine, Division of Nephrology and Arterial Hypertension, Zagreb, Croatia
(4) University Hospital Centre Zagreb, Division of Metabolic Diseases, Zagreb, Croatia

Corresponding author: tamara.bozina@mef.hr

Background: Metabolic syndrome (MS) is a cluster of modifiable risk factors including hypertension, abdominal obesity, dyslipidemia and insulin resistance, associated with nonmodifiable risk factors, such as age, sex and genetic background. We investigated the possible role of gene polymorphisms of *PPARG* (Pro12Ala), *ApoE* (ε2, ε3, ε4), *LPL* (P+/-), *IL-6* (-174G>C), *ACE* (I/D) and *AT1R* (1166A>C) in MS.

Materials and methods: 516 individuals were investigated including 263 patients with MS and 253 subjects without MS criteria. Genotyping was performed using PCR based methods.

Results: In female group associations were found for: LPL and ACE with MS (P = 0.04); *PPARG* and *LPL* with blood pressure, (P = 0.04); LPL with cholesterol and LDL (P = 0.01 and P = 0.05, respectively). Significant gene interactions observed between: *APOE* and *PPARG*, *ACE* and *APOE* were associated with BMI (P = 0.01 and P = 0.05, respectively); *LPL* and *PPARG* were associated with triglycerides (P = 0.03). For males we found associations of: *LPL* variants with MS (P = 0.02), BMI (P = 0.002) and waist circumference (P = 0.008); *PPARG* and *APOE* with BMI (P = 0.05); *IL-6* with CRP (P = 0.02). Significant gene interactions observed between: *PPARG* and *AT1R* were associated with blood pressure (P = 0.05); *PPARG* and *APOE* with triglycerides (P = 0.02); *PPARG* and *APOE*, *PPARG* and *IL6* (P = 0.03), *ACE* and *APOE* (P < 0.001) with cholesterol; *PPARG* and *LPL* (P = 0.003), *PPARG* and *IL6* (P = 0.06) with HDL; *PPARG* and *IL6* (P = 0.01), *ACE* and *APOE* (P = 0.04); *PPARG* and *APOE*, *LPL* and *ACE* (P = 0.01), *AT1R* and *ACE* (P = 0.06) with CRP.

Conclusion: Gene variants of *PPARG*, *APOE*, *LPL*, *ACE*, *AT1R* and *IL-6* could be susceptibility factors of obesity, lipid status, and glucose intolerance.

P15-10

Contribution of 5-*HTTLPR* and *BDNF* gene variants to obesity risk

Božina T (1), Sertić J (2), Lovrić J (1), Jelaković B (3), Jovanović N (4), Božina N (2)

(1) School of Medicine, University of Zagreb, Department of Medical Chemistry, Biochemistry and Clinical Chemistry, Zagreb, Croatia
(2) University Hospital Centre Zagreb, Department of Laboratory Diagnostics, Zagreb, Croatia
(3) University Hospital Centre Zagreb, Department of Internal Medicine, Division of Nephrology and Arterial Hypertension, Zagreb, Croatia
(4) University Hospital Centre Zagreb, Department of Psychiatry, Zagreb, Croatia

Corresponding author: tamara.bozina@mef.hr

Background: 5-Hydroxytryptamine (5-HT, serotonin) plays an important role in the central nervous control of energy balance and is involved in several biological processes including mood, appetite, sleep, libido, memory, and body weight regulation. Brain-derived neurotrophic factor (BDNF) is also currently recognized as an important participant in the regulation of food intake. The aim of this study was to evaluate whether the *5-HTTLPR* S/L and *BDNF* Val66Met gene variants are associated with obesity in a sample of adults of Croatian origin.

Materials and methods: 462 individuals were investigated including 301 obese (BMI ≥ 30 kg/m^2) and 161 lean (BMI < 25 kg/m^2) (mean age ± SD was 49 ± 8 years). Genotyping of triallelic structure of *5-HTTLPR* (LA, LG, S) and of *BDNF* Val66Met polymorphisms was performed using the RealTime-based allele specific PCR methods.

Results: In the whole group we found no associations between *5-HTTLPR* S/L and *BDNF* Val66Met polymorphisms and obesity. When we compared male and female samples, we observed statistically significant differences in the distribution of *5-HTTLPR* genotypes: *5-HTTLPR* LALA genotype was more frequent in the group of lean women comparing to the group of lean men (41% and 28% respectively, P = 0.022). The *5-HTTLPR* S and *BDNF* Met allele carriers have higher risk to develop obesity (OR 2.07) than non carriers (P = 0.038).

Discussion: Our findings indicate that *5-HTTLPR* polymorphism may be linked with obesity in adult female population, reinforcing the role of the serotonin transporter as a risk factor for the obesity phenotype. *SERTPR* and *BDNF* gene interactions could additionally predispose to obesity risk.

P15-11

ABCG2 gene variant and fluvastatin adverse drug reactions in renal transplant recipients

Mirošević Skvrce N (1), Božina N (2), Pejnović L (2), Barišić I (3), Zibar L (4), Macolić-Šarinić V (1)

(1) Agency for Medicinal Products and Medical Devices, Zagreb, Croatia
(2) University Hospital Centre Zagreb, University of Zagreb School of Medicine, Department of Laboratory Diagnostics, Zagreb, Croatia
(3) University Hospital Centre Zagreb, University of Zagreb School of Medicine, Department of Nephrology, Arterial Hypertension and Dialysis, Zagreb, Croatia
(4) Clinical Hospital Center Osijek, University of Osijek School of Medicine, Department of Internal Medicine, Osijek, Croatia

Corresponding author: nikica.mirosevic@almp.hr

Background: Polymorphisms in genes encoding drug transporters could be valuable pharmacogenetic markers. The *ABCG2* efflux transporter is expressed in multiple tissues and plays an important role in the disposition of different drugs including statins. The functional 421C>A polymorphism in the *ABCG2* that reduces transporter activity has been found to be associated with increased systemic exposures to certain statins, including fluvastatin. Although genetic variability in the *ABCG2* distinctively affects the pharmacokinetics, there are no published data that would indicate that variability can result in fluvastatin induced adverse drug reactions (ADRs). The aim of this case-control study is to show the contribution of pharmacogenetic predisposition (*ABCG2* gene variant) to the development of fluvastatin ADRs (myotoxicity, hepatotoxicity, other side effects) in renal transplant recipients.

Patients and methods: 108 renal transplant recipients were included in the study, 54 patients with ADRs to fluvastatin therapy, and 54 controls without ADRs (matched according to fluvastatin dose, age, gender, concomitant therapy, and other conditions). Genotyping of the *ABCG2* 421C>A polymorphism was performed using the TaqMan allele-specific PCR assay (AppliedBiosystems).

Results: According to the statistical analysis, *ABCG2* 421CA genotype carriers have significantly higher incidence of ADRs to fluvastatin therapy comparing to *ABCG2* 421CC genotype carriers (OR 3.77, 95%CI 1.26-11.28, P = 0.024).

Conclusion: Our data are the first to indicate there is an association between adverse drug reactions to fluvastatin therapy in renal transplant recipients with *ABCG2* 421C>A polymorphism.

P15-12

Association of soluble and -2518 A>G *CCL2* polymorphism with inflammation markers and IR

Guzman Ornelas M (1), Valdez Alvarado E (1), Aguilar Aldrete M (2), Sierra Amor R (3), Castro Albarran J (4), Navarro Hernandez R (5)

(1) Universidad de Guadalajara, Doctorado en Ciencias Biomedicas, Centro Universitario de Ciencias de la Salud., Guadalajara, Mexico
(2) Universidad de Guadalajara, Salud Publica, Centro Universitario de Ciencias de la Salud., Guadalajara, Mexico
(3) Iniciativa Privada, Laboratorios LAB-QUIM, Veracruz, Mexico
(4) Secretaria de Salud Jalisco, Nutricion, H.M.I.E.L.M., Guadalajara, Mexico
(5) Universidad de Guadalajara, Biologia Molecular y Genomica, Centro Universitario de Ciencias de la Salud., Guadalajara, Mexico

Corresponding author: rosa_elena_n@hotmail.com

Introduction: Insulin resistance (IR) is a disease with genetic susceptibility characterized by an abnormal inflammatory response. CCL2 is secreted by adipocytes, plays a central role in macrophage accumulation in white adipose tissue (WAT), promoting the inflammatory process, remain suggested to be involved in the progression of obesity to IR. The *CCL2* gene is overexpressed in WAT and decreases insulin-stimulated glucose uptake into adipocytes. The -2518A>G polymorphism in the regulatory region of *CCL2* may regulate gene expression. The aim was to investigate the relationship of *CCL2* -2518A>G polymorphism and sCCL2 with inflammation markers and IR.

Materials and methods: In a cross-sectional study we included 309 individuals Mexican-mestizo, classified by HOMA-IR index. Body composition, anthropometrics and inflammation markers were measured by routine methods, and -2518A>G variants by PCR-RFLP and sCCL2 by ELISA methods.

Results: In this study group we found differences in: 1) sCCL2 levels (191 ± 14.9, 280 ± 21.6 ng/mL, P = 0.001); and 2) the genotype frequencies (AA: 33%, 30%; GA: 53%, 41% and GG: 14%, 29%, P = 0.007) between healthy and IR individuals, respectively. The G allele carriers showed lower measures (101 ± 9.7 cm) of hip circumference than the A allele carriers (104 ± 11.4 cm). While in IR individuals, the GG genotype carriers showed higher levels of: CRP, WBC and triceps skin fold thickness than the GA+AA genotype carriers. The sCCL2 levels showed correlations with CRP, glucose, sInsulin, HOMA-IR, weight, BMI and hip-circumference (r = 0.190 to 0.350, P < 0.05).

Conclusions: We suggest that CCL2 allele -2518G is associated with inflammatory course and distribution of body fat in IR Mexican-mestizo.

P15-13

Association of PAI-1 5G allele with inflammation markers and body fat in Mexican with obesity

Diaz-Rubio G, Navarro Hernandez R, Chavarria-Avila E, Aguilar-Uscanga B, Reyes-Blanco M, Ruiz-Quezada S

Universidad de Guadalajara, Departamento de Farmacobiologia, Centro Universitario de Ciencias Exactas e Ingenierias, Guadalajara, Mexico

Corresponding author: sandyruizq2005@yahoo.com.mx

Introduction: Comorbidity in obesity are diseases with genetic susceptibility and characterized by an inflammatory response. An association between obesity, inflammation and reduced fibrinolysis activity contributes to a higher risk of cardiovascular events. The plasminogen activator inhibitor-1 (PAI-1) is the main inhibitor of fibrinolysis, representing approximately 60%. The sources of PAI-1 are endothelium cells, platelets, adipocytes and stromal cells of adipose tissue. The gene variants of PAI-1 are associated with cardiovascular diseases. Our aim was to investigate the relationship of *PAI-1* 4G/5G polymorphism with inflammation markers and body fat distribution in obesity.

Materials and methods: In a cross-sectional study we included 179 individuals Mexican-mestizo, classified by BMI index. Body composition, anthropometrics and inflammation markers were measured by routine methods, 4G/5G polymorphism by PCR-RFLP, and insulin by ELISA methods.

Results: In this study group we found differences in hsCRP levels (1.87 ± 0.36, 4.73 ± 1.64 mg/L, P = 0.031); and body-fat-index (2.17 ± 0.07, 2.54 ± 0.18, P = 0.032) between 5G/5G and 4G/4G genotype carriers. The hsCRP levels showed correlations with distribution and body fat mass (r = 0.259 to 0.557, P < 0.05). While, the genotype frequencies were (Lean: 16%, 41%, 43%; overweight: 12%, 48%, 40%; obese: 16%, 58%, 26%) for 4G/4G, 4G/5G and 5G/5G genotype carriers, respectively. In individuals classified as obesity, we found the following differences: total body fat (43% *vs.* 34%; P = 0.015), waist-hip-ratio (0.8710 ± 0.18 *vs.* 0.9328 ± 0.18; P = 0.024) and platelet-count (237.200 ± 10.595 *vs.* 288.500 ± 15.743; P = 0.010) in 5G/5G *vs.* 4G/5G + 4G/4G genotype carriers.

Conclusions: We suggest that 4G/5G *PAI-1* polymorphism may be associated with inflammatory process and body fat distribution in Mexican-mestizo individuals with obesity.

P16 - Molecular diagnosis 2

P16-01

Genotyping of 13 α-thalassaemia point mutations using primer extension reaction and dipstick assay

Petropoulou M

National and Kapodistrian University of Athens, Department of Chemistry, Athens, Greece

Corresponding author: mpetrop@biol.uoa.gr

Background: Alpha-thalassaemia is inherited as an autosomal recessive disorder characterized by a clinical phenotype varying from almost asymptomatic to a lethal hemolytic anemia. Reduced or absent a-globin synthesis, mainly caused by deletions of one or both a-globin genes and by point mutations, leads to a-thalassemia (a-thal). Numerous techniques have been developed for the identification of the underlying genetic defect in affected individuals.

Materials and methods: The method consists of: (i) PCR amplification of a single fragment (1087 bp) of the a1 and a2 globin gene flanking all 13 mutations; (ii) three 10-plex primer extension reactions of the unpurified amplification product using allele-specific primers, carrying at their 5'end a unique recognition sequence. The reaction takes place in the presence of biotin-dUTPs and a DNA polymerase that lacks 3'→5' exonuclease activity and (iii) dry-reagent multi-allele dipstick assay for visual detection of the primer extension reaction products within minutes. The detection of the products is achieved by naked eye using anti-biotin conjugated gold nanoparticles.

Results and conclusions: Parameters that affect the performance of the PCR amplification, PEXT reaction and the multiallele biosensor were investigated in order to optimize the specificity and detectability of genotyping assay. The method was evaluated by analyzing 83 samples of known genotypes and the results were found to be fully concordant with those obtained by the reference methods. The proposed method is simple, rapid, cost-effective, does not require specialized instrumentation or purification of the PCR products and could be a particularly useful tool in laboratories with limited resources.

P16-02

Descriptive study of genotype frequencies of Ala9Val polymorphism of SOD-Mn in chagasic patients

D'arrigo M (1), Gerrard G (1), Lioi S (1), Zumoffen C (1), Beloscar J (2)

(1) Facultad de Ciencias Bioquimicas Y Farmaceuticas Universidad Nacional de Rosario, Bioquimica Clinica, Rosario, Argentina
(2) Facultad de Ciencias Medicas Universidad Nacional de Rosario, Cardiologia, Rosario, Anguilla

Corresponding author: darrigomabel@yahoo.com.ar

Introduction: The existence of individuals infected with Chagas disease (CD) living in endemic areas without apparent heart damage shows that a proportion of them are able to contrarest the infection by *Tripanosoma cruzi*. Genetic factors of each patient ere actively involved in the evolution of clinical manifestations of CD. In this work we decided to do a descriptive study of genotype frequencies (GF) of Ala9Val polymorphism of SOD-Mn and determine the enzyme activities of superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) in chagasic patients (CP) with cardiomyopathy (CwC) and without cardiomyopathy (CnC) compared with healthy controls (HC).

Materials and methods: The molecular characterization was performed by PCR-RFLP. Enzyme activities were determined by spectrophotometric methods. The hypothesis test under normal theory proportions and Kruskal Wallis test were carried out.

Results: The GF of SOD-Mn (IC 95%) were: HC: Ala/Ala: 0.54, Ala/Val: 0.33, Val/Val: 0.13; CwC: Ala/Ala: 0.35, Ala/Val: 0.30, Val/Val: 0.35; CnC: Ala/Ala: 0.36, Ala/Val: 0.46, Val/Val: 0.18. The enzyme activities

were: CAT (K/gHb): HC: 185 ± 28, CwC: 316 ± 68, CnC: 332 ± 41; GPx (U/gHb): HC: 61 ± 1, CwC: 98 ± 17, CnC: 102 ± 20; SOD (USOD/gHb): HC: 895 ± 314, CwC: 3270 ± 833, CnC: 2590 ± 188. The study of SOD-Mn GF and the activities of CAT, SOD and GPx showed significant differences (P<0.01) between CP and HC.

Conclusion: The data reflect that polymorphisms involved in oxidative stress may have implications in the pathogenesis of CD, modifying individual risk in the development of cardiomyopathies.

P16-03

Endothelial NO-synthase gene polymorphism in pathogenesis of lacunar stroke

Topuzova MP (1), Klocheva EG (1), Zaytseva NO (2), Goldobin VV (1), Vavilova TV (2), Sirotkina OV (2)

(1) North-West State Medical University named after Mechnikov, Neurology, St. Petersburg, Russia (Russian Fed.)
(2) North-West State Medical University named after Mechnikov, Clinical laboratory diagnostics, St. Petersburg, Russia (Russian Fed.)

Corresponding author: marcun@rambler.ru

Objective: Determination of endothelial NO-synthase T786S and G894T eNOS polymorphisms in patients with microangiopathy in acute period of ischemic lacunar stroke (LS).

Materials and methods: We examined 67 patients with LS due to microangiopathy (54% female, 46% male, mean age 66 ± 11) and 45 healthy controls. The diagnosis of LS was verified in accordance to current diagnostic criteria, clinically and instrumentally. Genotypes were determined by RT-PCR using the amplifier DT-96 and reagent kit "DNA-Technology" (Moscow, Russia).

Results: The analysis of T786C and G894T eNOS showed the following genotypes distribution: TT in 37.3%, CT in 55.2% and CC in 7.5% patients; GG – 55.2%, GT – 38.8% and TT in 6% patients with LS. Investigation of T786C and G894T eNOS in controls revealed the following genotypes frequencies: TT – 46.7%, CT – 44.4% and CC – 8.9%; GG – 71.1%, GT – 22.2% and TT – 6.7%, respectively. The distribution of T786C eNOS genotype was not significantly different between patients with LS and controls. While, the significant difference was observed in distribution of the G894T eNOS genotypes (P = 0.03). Thereby, the presence of T allele of the G894T eNOS in the homo-or heterozygous state was a risk factor for LS (OR=1.99; CI = 1.07-3.74).

Conclusion: The prevalence of 894T allele *eNOS* gene in patients with microangiopathy in acute period of ischemic stroke suggests an association of carriage of this allele and development of LS.

P16-04

Clinical manifestation of hereditary angioedema is less severe in patients with missense mutations

Bors A (1), Varga L (2), Farkas H (2), Tordai A (1), Fust G (2), Szilagyi A (2)

(1) Hungarian National Blood Transfusion Service, Molecular Diagnostics Laboratory, Budapest, Hungary
(2) Semmelweis University, 3rd Department of Internal Medicine, Budapest, Hungary

Corresponding author: bors.andras@ovsz.hu

Background: Hereditary angioedema (HAE) is a rare (1:50000), but potentially life-threatening disease, characterized by recurrent, acute edematous episodes, and caused by C1-inhibitor (C1INH) deficiency with an autosomal dominant inheritance. The rate, localization, and severity of symptoms are highly variable among patients. Our aim was to evaluate the genotype-phenotype correlation between disease-causing mutations in C1INH gene and the diverse clinical manifestation of HAE.

Patients and methods: Screening for C1INH mutations was carried out by DNA sequencing, Southern-hybridisation and qPCR based on relative quantification of each exon in 39 unrelated Hungarian HAE type I patients. Patients were followed for a minimum of 5 years and annual frequencies of attacks (subcutaneous, abdominal, upper air-

way, genital, lingual-labial, erythema) were recorded.

Results: In summary, 7 missense, 4 nonsense, 3 splice site mutations, 1 point mutation in the translation initiation codon, 4 large deletions, 1 small deletion, and 7 frameshift (including 4 previously not reported) mutations were identified. No point mutation or large gene rearrangements were detectable in 6 patients. Annual frequency of attacks, and requirement of C1-inhibitor replacement was significantly lower in carriers of missense mutations (N = 7) compared to patients with non-missense mutations (N = 32, P = 0.008 and P = 0.022, respectively). Carriers of missense mutations had 10 times lower odds ratio for high (> 1/year) attack frequency compared to the rest of patients.

Conclusions: Our findings indicate that missense mutations of the C1INH gene are associated with favourable manifestation of the disease. Information on the mutation type can be used in clinical practice for predicting the severity of HAE.

P16-05

Serotonin transporter polymorphism (*5-HTTLPR*) in Croatian population

Culej J (1), Štefanović M (2), Karlović D (3)

(1) Sestre Milosrdnice University Hospital, Department of transfusiology and haemostasis - Clinic for tumors, Zagreb, Croatia
(2) Sestre Milosrdnice University Hospital, Clinical Institute of Chemistry, Zagreb, Croatia
(3) Sestre Milosrdnice University Hospital, Department of Psychiatry, Zagreb, Croatia

Corresponding author: jelena.culej@gmail.com

Background: Serotonin transporter is responsible for serotonin reuptake in the brain as well as other tissues. The most studied polymorphism is consisted of 44 base pairs deletion/insertion in the promoter region (*5-HTTLPR*) that classifies alleles into „short" (S) or „long" (L) variant. Short allele is associated with reduced transcriptional activity. Some previous family based studies support thesis of genetic basis of psychiatric disorders. According to previous research, this polymorphism is related to depression and other psychiatric disorders, as well as with response to selective serotonin reuptake inhibitors treatment. Genetic heterogeneity of population might cause misleading association between cases and controls in case-control studies. The aim of this study was to assess frequency of S and L alleles in Croatian population.

Materials and methods: 307 healthy individuals of both sexes, (age 18 to 50) were included in this study. Their physical and psychical condition was assessed by physical examination, and MINI psychiatric interview for mental disorders exclusion. Genotyping was performed by simple PCR reaction followed by the gel electrophoresis which is used for separation of 375 bp (allele S) from 419bp (allele L).

Results: Genotype frequencies were in accordance with Hardy-Weinberg equilibrium (P = 0.114). L allele frequency is 59% and for S allele 41%. Frequency distribution for studied genotype is: LL – 37%; LS – 44%; SS – 19%.

Conclusion: Based on Hardy – Weinberg equilibrium, investigated population is homogenous and reported genotype frequencies are in accordance to some other studies performed on European population.

P16-06

Q192R and L55M pon1 gene polymorphisms associated with peripheral arterial disease

Kardum Paro M (1), Perkov S (1), Flegar- Meštrić Z (1), Vidjak V (2), Novačić K (2), Barišić K (3)

(1) Merkur University Hospital, Institute of Clinical Chemistry and Laboratory Medicine, Zagreb, Croatia
(2) Merkur University Hospital, Institute for Radiology, Zagreb, Croatia
(3) University of Zagreb, Faculty of Pharmacy and Medical Biochemistry, Zagreb, Croatia

Corresponding author: mirjanamariana@gmail.com

Background: Development of peripheral arterial disease (PAD) could be due to human serum paraoxonase (*pon1*) gene polymorphisms or lower catalytic concentrations of serum paraoxonase-1 (PON1). The aim of study was to examine the association of *pon1* gene polymorphisms (Q192R and L55M) with the catalytic concentrations of serum PON1 in patients with PAD.

Materials and methods: Healthy subjects (118) and patients with angiographically confirmed diagnosis of PAD (110) were investigated. The catalytic concentration of serum PON1 was determined by spectrophotometric method using paraoxon as the supstrate in the presence of NaCl. *pon1* gene polymorphisms were determined by PCR-RFLP method accredited according to ISO15189. The assay performance was assessed by interlaboratory specimens exchange. Allele and genotype frequencies were compared by the $\chi 2$ or Fisher exact test.

Results: For *pon1* gene polymorphism Q192R allele and genotype frequency were significantly different betweeen patients with PAD and healthy subjects (both < 0.001), while for *pon1* gene polymorphism L55M allele (P = 0.649) and genotype (P = 0.327) frequencies did not differ significantly. The catalytic concentrations of serum PON1 were significantly lower in patients with PAD (P = 0.001). In both groups studied the lowest catalytic concentration were found in QQ and MM genotypes of *pon1* gene.

Conclusions: Lower catalytic concentrations of serum PON1 and Q192R *pon1* gene polymorphism could play a role in the development of PAD. Differences in the Q and R allelle frequencies between healthy subjects and patients with PAD may be one of the causes for the reduced catalytic concentrations of serum PON1.

P16-07

Platelet receptor for von Willebrand factor: gene polymorphism and cerebrovascular disease

Laskovets A (1), Zaytseva N (1), Goldobin V (2), Klocheva E (2), Vavilova T (1), Sirotkina O (3)

(1) North-West State Medical University named after Mechnikov, Clinical Laboratory Diagnostics, St-Petersburg, Russia (Russian Fed.)
(2) North-West State Medical University named after Mechnikov, Neurology, St-Petersburg, Russia (Russian Fed.)
(3) B.P. Konstantinov Petersburg Nuclear Physics Institute, Human Molecular Genetics, St-Petersburg, Russia (Russian Fed.)

Corresponding author: laskovetsanas@mail.ru

Objective: Platelet activation and aggregation are pivotal in the pathogenesis of cerebrovascular disease. The aim of our study was to determinate the von Willebrand (vWF) factor platelet receptor gene polymorphism in patients with different pathogenetic variants of stroke.

Materials and methods: We examined the T(-5)C and Thr145Met GP Iba in 47 healthy controls and 123 patients with stroke due to macroangiopathy (1 group), microangiopathy (2 group) and subjects with pathological tortuosity of brachiocephalic arteries, but without ischemic stroke (3 group) by PCR-RFLP.

Results: The analysis of T(-5)C GP Iba showed the following genotypes distribution: 75.6% and 24.4%; 65.9% and 34.1%; 82.9% and 17.1%; 78.7% and 21.3% for TT and TC in 1, 2, 3 and control groups, respectively. There was no CC genotype in investigated subjects. The distribution of T(-5)C GP Iba genotype between patients and controls was not significantly different. However, the significant difference was observed in the Thr145Met GP Iba

genotypes' frequencies – 73.2%, 22.0%, 4.8% and 89.4%, 10.6%, 0% for ThrThr, ThrMet and MetMet in 1 group and controls, respectively (P = 0.045). The Thr145Met GP Iba genotypes' frequencies were not significantly different in 2 and 3 groups – 87.8%, 9.8%, 2.4% and 90.2%, 9.8%, 0%. Thereby, the presence of the 145Met GP Iba allele in the homo-or heterozygous state was a risk factor for stroke due to macroangiopathy (OR = 3.08 [95% CI 1.02-9.33]).

Conclusion: The prevalence of the Thr145Met GP Iba in patients with macroangiopathy suggests an association of carriage of this allele and the development of ischemic stroke.

P16-08

Li-Fraumeni Syndrome: description of a new pathogenic TP53 mutation

Illana F (1), Llovet P (2), de la Hoya M (2), Olivera H (3), Perez-Segura P (3), Caldes T (2)

(1) Hospital Clinico San Carlos, IdISSC, Laboratory Medicine Department, Madrid, Spain
(2) Hospital Clinico San Carlos, IdISSC, Molecular Oncology Laboratory, Madrid, Spain
(3) Hospital Clinico San Carlos, Department of Medical Oncology, Madrid, Spain

Corresponding author: fran_illana@hotmail.com

Background: Li-Fraumeni syndrome is associated to germ-line mutations of one TP53 allele. The most common cancers associated with LFS are breast cancer (30.6%), soft tissue sarcomas (17.8%), brain tumors (14%) and osteosarcome (13.4%). In this work, we found a novel germline TP53 mutation in a high risk breast/ovarian cancer family that's not reported in the P53_IARC database.

Material and methods: We studied *BRCA1* and *BRCA2* genes in blood of a high risk breast/ovarian family by Denaturing Gradient Gel Electrophoresis and Multiplex Ligation-dependent Probe Amplification. Also, exons 5 to 9 and splice junctions of TP53 were sequenced in blood and tumors of two relative's patients (30 and 32 years old) who suffered breast cancer (one of them was a Cystosarcoma Phyllodes). Microsatellites were studied in these tumors too.

Results: The whole study of *BRCA1* and *BRCA2* genes was negative for mutations and deletions/insertions. Sequences of TP53 in germ-line showed a deletion of 9 nucleotides in exon 5 (c.436del9), but it maintains the open reading frame. The deletion is located in binding DNA domain. However, not deletereus effect has been predicted in silico analysis. The study of the TP53 protein by immunohistochemistry (IHC) in the Cystosarcoma Phyllodes showed high expression of the protein. Microsatellites and the sequencing of TP53 in tumors showed the loss of the normal allele (LOH).

Conclusions: The positive IHC, the segregation of the variant with the disease and the LOH of wild type allele in this family indicates that new variant c.436del9; p.Trp146_Asp148del would be pathogenic.

P16-09

ATP7B gene mutations in Wilson disease patients from Croatia

Ljubić H (1), Božina T (2), Merkler A (1), Relja M (3), Kalauz M (4), Sertić J (1)

(1) University Hospital Centre Zagreb, Department of Laboratory Diagnostics, Zagreb, Croatia
(2) School of Medicine University of Zagreb, Department of Medical Chemistry, Biochemistry and Clinical Chemistry, Zagreb, Croatia
(3) University Hospital Centre Zagreb, Department of Neurology, Zagreb, Croatia
(4) University Hospital Centre Zagreb, Department of Internal Medicine, Zagreb, Croatia

Corresponding author: hljubic@kbc-zagreb.hr

Background: Wilson disease (WD) is a rare autosomal recessive disorder of copper metabolism associated with progressive liver disease and degenerative changes in the basal ganglia. More than 400 mutations of the ATP7B gene have been proven to cause the absence or dysfunction of copper transporting P-type ATPase. If WD is diagnosed early enough, effective treatments are available that prevent or reverse many manifestations of this disorder.

Materials and methods: 84 unrelated patients, with hepatic, neurological and/or psychiatric symptoms. Biochemical analyses considering copper levels in serum, urine and liver were indicative of WD diagnosis. Genomic DNA was used to amplify 21 exons of the ATP7B gene. Sequencing analysis was performed by PCR and capillary electrophoresis with BigDye Terminator v3.1 kit on Applied Biosystems Genetic analyzer 3130xl. Preliminary screening for most frequent His1069Gln mutation was performed on Roche LightCycler real-time PCR instrument.

Results: Out of total number of WD patients, molecular-genetic analysis confirmed clinical diagnosis in 41 patient (48.8%). 19 (22.6%) patients were found to be heterozygous for certain WD mutation, while in 24 patients (28.6%) no mutations were found. The most frequent mutation in Croatian population is His1069Gln in exon 14 of *ATP7B* gene, which accounts for 63.3% of total number of WD mutations. Other frequent mutations are mostly located in exons 5, 13, 15 and 21 of *ATP7B* gene.

Conclusions: Sequencing analysis is the most adequate method for diagnostics of Wilson disease because of its genetic heterogeneity and also in cases with atypical clinical presentation or equivocal copper studies.

P16-10

Molecular genetic analysis of primary immunodeficiency diseases in Croatian patients

Merkler A (1), Richter D (2), Kelečić J (2), Rako I (1), Marodi L (3), Sertić J (1)

(1) University Hospital Center Zagreb, Department of Laboratory Diagnostics, Zagreb, Croatia
(2) University Hospital Center Zagreb, Department of Pediatrics, Zagreb, Croatia
(3) University of Debrecen, Department of Infectious and Pediatric Immunology, Debrecen, Hungary

Corresponding author: amerkler@kbc-zagreb.hr

Background: Primary immunodeficiency diseases (PID) are a group of inherited conditions that occur in individuals born with malfunctioned immune system. The aim was to confirm clinical diagnosis at molecular genetic level in patients with PID and to define carrier status by analyzing DNA samples of individuals with PID in family history.

Materials and methods: 15 samples of genomic DNA was analyzed: 7 samples of patients with suspicion on one of the PID and 8 samples of their family members. For identification of mutations in the coding region of analyzed genes, we used the sequencing method on Applied Biosystems 3130xl Genetic analyzer and BigDye® Terminator v3.1 Cycle Sequencing Kit.

Results: In 2 patients with cyclic and severe congenital neutropenia, we found 2 mutations occurred de novo in mother's egg cells. In a patient with suspicion on Omenn syndrom, mutation wasn't found. In 2 patients with X-linked agammaglobulinemia, mutations in the BTK gene were found. In the first patient, mutation occurred de novo in mother's egg cells and, in the other, mutation was inherited from the mother. The third patient with XLA suspicion did not have mutation in the BTK gene. In a patient with suspicion on chronic granulomatous disease, mutation wasn't found, so there is a possibility for further analysis of other genes associated with this disease.

Conclusions: Molecular genetic analysis is the final diagnostic step in confirmation of the PID diagnosis as it provides comprehension of disease mechanisms at the molecular level and correlation between phenotype and genotype of PID.

P16-11

HLA-DQ genotyping of celiac disease in Mersin Province of Turkey

Polat G (1), Yesiltepe Y (1), Altıntas E (2), Ates F (2)

(1) Mersin University, Faculty of Medicine, Department of Medical Biochemistry, Mersin, Turkey
(2) Mersin University, Faculty of Medicine, Department of Gastroenterology, Mersin, Turkey

Corresponding author: gurbuzp@gmail.com

Background: Celiac disease is a common autoimmune disease caused by a chronic inflammatory reaction to the dietary protein gluten in the intestine. It is characterized by gluten-induced symptoms and signs, specific antibodies, specific HLA class II types and enteropathy. The objective of this study is to determine the genetic profile of celiac disease in Mersin Province of Turkey.

Materials and methods: HLA-DQ2, -DQ8 and -DR4 molecular typing was performed in 220 patients. The molecular typing was analyzed using polymerase chain reaction sequence-specific primers and/or reverse polymerase chain reaction sequence-specific oligonucleotides techniques.

Results: We have found HLA-DQ2, -DQ8 and -DR4 types or their combinations in 133 patients. 27.7% of all patients (61/220) were HLA-DQ2, 3.1% (7/220) were -DQ8 and 8.6% (19/220) were -DR4. The combinations were 10.4%, 5.9%, 3.6% and 0.9% for – DQ8 + DR4, DQ2 + DQ8 + DR4, DQ2 + DQ8 and DQ2 + DR4, respectively. 45.8 % of patients showing polymorphisms (61/133) were HLA-DQ2, 5.2% (7/133) were -DQ8 and 14.2% (19/133) were -DR4. The combinations were 17.3%, 9.77%, 6.0% and 1.5% for DQ8 + DR4, DQ2 + DQ8 + DR4, DQ2 + DQ8 and DQ2 + DR4, respectively.

Conclusion: These results support the evidence that HLA-DQ status influences the development of celiac disease. Further, determination of patient's HLA-DQ genotype allows establishing clinically relevant genetic risk profiles.

P16-12

Sequencing analysis of *CFTR* gene in Croatian patients with cystic fibrosis

Zekušić M (1), Ljubić H (1), Juričić L (1), Tješić-Drinković D (2), Rako I (1), Sertić J (1)

(1) University Hospital Centre Zagreb, Department of Laboratory Diagnostics, Zagreb, Croatia
(2) University Hospital Centre Zagreb, Department of Pediatrics, Zagreb, Croatia

Corresponding author: mzekusic@kbc-zagreb.hr

Background: Cystic fibrosis (CF) is the most common autosomal recessive hereditary disease in Caucasians with an average incidence of 1:2500 newborns. *CFTR* gene codes for CFTR protein (cystic fibrosis transmembrane conductance regulator) which regulates the transport of chloride ions through cell membranes.

Materials and methods: Genomic DNA of 65 CF patients was screened for 32 mutations by Cystic Fibrosis Genotyping Assay CFv3 (Abbott). Twelve patients who were found to be heterozygous for one CF mutation were further analysed by sequencing method for specific exons (7, 10, 11, 12 and 19) on AB Genetic Analyzer 3130XL, using BigDye Terminator Cycle Sequencing Kit v3.1 (Applied Biosystems).

Results: Since year 2001, out of 65 patients detected to have CF, 42 were homozygous with the deltaF508 mutation and 11 were compound heterozygous. By using commercial kit we revealed 14 different mutations in patients with CF. Through sequencing exons 7, 10, 11, 12 and 19 in 12 heterozygous patients so far we have found five polymorphisms: M470V (c.1408A>G), 1898+152T/A (c.1766+152T>A), 3601-65C/A (c.3469-65C>A), 1525-61A/G (c.1393-61A>G) and R75Q (c.224G>A). These DNA polymorphisms will certainly contribute to understanding the sequential variants and clinical correlation. Other *CFTR* exons still remain to be analyzed.

Conclusions: This paper investigates variants in *CFTR* gene other than the panel covered by commercial kit. By collecting information about similar

cases and studying them, we are likely to notice a link between new mutations, polymorphisms and clinical features.

P17 - Oncology - Tumor marker 1

P17-01

New strategies to improve the quality and efficiency of health assistance to chronic cancer patients

Torrubia B, Guessous A, De Gracia Y, Cuadrado-Cenzual M

Hospital Clinico, Analisis Clinicos, Madrid, Spain

Corresponding author: mcuadrado.hcsc@salud.madrid.org

Background: Integral care to the cancer patient, has a relevant social impact while it requires a multidisciplinary vision of the different areas of medicine to the patient care. Patients in oncological units are the most common class of potentially difficult draws because they are subject to more frequent laboratory testing. It is necessary to evaluate how care is applied to cancer patients and to develop strategies for improvement oncological care process. The aim of the present work was to evaluate of the health care process of the cancer patient and to improve strategies aimed at incorporating the patients' prospects to the care provision measures.

Materials and methods: Development of two care models: 1º: High Resolution Model (MCAR) that include analytical process and the administration of the therapy at the hospital in the same morning; and 2º: Accessibility Analytics Patient Model (MAAP) improve the accessibility to the patients at the analytical tests, making possible the access to different flebotomy services points.

Results: The percentage of cancer patients who are in treatment has undergone a biannual 8.76% increase. MCAR are used by 58% oncology patients. In relation to MAAP model there was an increase in the use of this model up to 42% in the two years of its implementation.

Conclusion: The optimized and preferred care circuits implementation, in both models have proven to be safe, effective, improving the accessibility of patients to diagnostic testing.

P17-02

Usefulness of inflammatory markers in assessing the severity of colorectal cancer

Đerek L (1), Servis D (2), Unic A (1), Juricek J (1), Romic Z (1)

(1) University Hospital Dubrava, Clinical Department for Laboratory Diagnostics, Zagreb, Croatia
(2) University Hospital Dubrava, Department for Abdominal Surgery, Zagreb, Croatia

Corresponding author: lderek@kbd.hr

Background: Severe abdominal surgeries, including colorectal cancer (CRC) surgery lead to systemic inflammatory response, which is followed by the increase of inflammatory markers. Intensity of inflammatory response follows the severity of surgical injury and influences postoperative recovery. Considering that the duration of the surgery, as well as the time that the colon has been open during the surgery (open colon time, OCT) define the severity of CRC surgery, and consequently influence the postoperative recovery, we wanted to investigate if the inflammatory markers correlate with those two factors and thus provide useful information.

Materials and methods: The study included 20 patients who underwent CRC surgery. Duration of the surgery, and OCT were measured for the duration of the procedure. C-reactive protein (CRP), interleukine-6 (IL-6), ferritin and soluble triggering receptor expressed on myeloid cells-1 (sTREM-1) were measured 24 hours after the surgery. CRP and ferritin were measured using immunoturbidimetric method (Beckman-Coulter, Tokyo, Japan), IL-6 and sTREM-1 using ELISA method (Quantikine, R&D Systems, Minneapolis, USA; IQ products BV, Groningen, The Netherlands).

Results: Statistical analysis showed no correlation between tested markers and the duration of the surgery. We found strong correlation for IL-6 ($r = 0.8147$, $P < 0.001$) with OCT. No correlation between CRP, ferritin and sTREM-1 and the OCT was found.

Conclusions: Our results showed that CRP, ferritin and sTREM-1 do not point to the injury and recovery after CRC surgery. We found strong positive correlation of IL-6 with the OCT during the surgery, so the rise of IL-6 could provide useful information in following CRC surgery severity and postoperative recovery.

P17-03

The phosphorescence as a predictive basis of early diagnosis of oncopathology

Gramatiuk S

Kharkiv National Medical University and Medical and diagnostic center 'Ekomed', Clinical biochemistry, Kharkiv, Ukraine

Corresponding author: gramatyuk@ukr.net

Serum proteins phosphorescence state was studied and evaluated in 59 patients with stomach adenocarcinoma, aged from 35 to 68 years. Diagnosis was confirmed by clinical and histomorphological methods. Among patients with gastroadenocarcinoma phosphorescence was studied in 33 men and 26 women. I, II, III and IV stages of disease were detected in 8, 7, 9 and 9 men and 6, 7, 7, and 6 women, accordingly. The study of luminol-dependent serum films phosphorescence in patients with adenocarcinoma of the stomach revealed increasing in men at 94.5%, 41.9%; 44.5%, 73.95%, 286.8% and 217.9% at such activation spectral lines as 297, 313; 334, 365, 404 and 434 nm. In women, the intensity of phosphorescence in similar lines of activation with monochromatic light of 297, 313, 334, 365, 404 and 434 nm, rised up to 93.2%, 33.7%, 29.6%, 67.4%, 286.7% and 216.7%. The mostly significant indexes of phosphorescence were detected at activation with monochromatic light of 297 nm, 404 nm and 434 nm. Serum phosphorescence intensity in patients at activation with monochromatic light of 297 nm wavelength rised up by 1.94 and 1.93 times accordingly in men and women in comparison with a group of conditionally healthy people. At activation with wavelength of 404 nm serum phosphorescence in patients increased by 3.7 and 3.6 times, at 434 nm by 3.2 and 3.1 times in comparison with a group of conditionally healthy people.

P17-04

Study of cytokines, as potential biomarkers in the diagnosis of oral squamous cell cancer

Hamad A

Al-Balqa Applied University, Medical Allied of Science, Amman, Jordan

Corresponding author: wahabhamad2004@yahoo.com

Introduction: Oral cancer is one of the prevalent cancers of the body and is one of the 10 most common causes of death. Oral squamous cell carcinoma (OSCC) accounts for over 90% of these tumors. The aim of this study was designed to detect biochemical markers in serum and saliva of oral squamous cell carcinoma patients and to evaluate their validity in monitoring and diagnosis.

Materials and methods: The level of certain pro-inflammatory cytokines in the serum and saliva of (30) patients with OSCC and (20) healthy individuals as control group was measured. Levels of pro-inflammatory cytokines Interleukin 1" (IL-1"), Interleukin (IL-6), Interleukin (IL-8) and Granulocyte Macrophage-Colony Stimulating Factor (GM-CSF) was detected by enzyme linked immunosorbent assay (ELISA).

Results: Serum IL-6 and IL-8 level was detected at higher concentrations in patients with OSCC than the control group ($P < 0.001$). No significant differences in serum IL-1 alpha and GM-CSF of patients with OSCC as compared with control group.The levels of IL-1 alpha, IL-6, IL-8 and GM-CSF in saliva

showed significant increase in patients with OSCC when compared with control group.

Conclusion: Salivary IL-1 alpha and GM-CSF was useful in the diagnosis of OSCC patients. Serum IL-6 was useful in the diagnosis of OSCC patients than salivary IL-6. Serum and salivary IL-8 were very useful in the diagnosis of OSCC patients and separating between OSCC patients and control group. From the results of the presents study, it can be concluded that cytokines are important in proinflammatory and proangiogenic responses and are detectable in serum and saliva of patients with OSCC. These cytokines increase the pathogenicity of OSCC and prove useful as biomarkers for diagnosis.

P17-05

Germline polymorphisms in LRIG1 gene predict clinical outcome in metastatic colorectal cancer

Paez D (1), Pare L (2), Sebio A (2), Del Rio E (2), Barnadas A (1), Baiget M (2)

(1) Hospital de la Santa Creu i Sant Pau, Medical Oncology, Barcelona, Spain
(2) Hospital de la Santa Creu i Sant Pau, Genetics, Barcelona, Spain

Corresponding author: mbaiget@santpau.cat

Background: Leucine-rich and immunoglobulin-like domains (LRIG) 1, 2, and 3 are integral membrane proteins. LRIG1 negatively regulates EGF signaling and increasing evidence indicates that LRIG1 is a tumor suppressor in certain cancer types. Lrig1 is expressed at low levels in several cancer types but is overexpressed in some colorectal tumors. We postulate that polymorphisms in the *LRIG1* gene could influence the EGFR signalling pathway and be related with the clinical outcome in metastatic colorectal cancer (mCRC).

Materials and methods: We studied 126 Spanish mCRC treated with a first-line oxaliplatin/5-fluorouracil regimen. Polymorphisms in the LRIG gene were selected using public literature resources and databases (NCBI, PubMed, db SNP, Ensembl and GeneCards Version 3). Two non-synonymous (c3158A>C and c1843A>G) and two synonymous (c2221T>C and c1317C>T) common and putatively functional polymorphisms were selected. These germline polymorphisms were analyzed using a Fluidigm equipment in DNA samples extracted from peripheral blood. Overall survival (OS) was evaluated according to each genotype.

Results and conclusions: There were significant associations between *LRIG1* c1317C>T and c3158A>C polymorphisms and clinical outcome. The analysis of the c1317C>T polymorphism revealed that OS was lower for patients harboring a T/T genotype than for patients with the C/C or C/T genotypes (P = 0.01). In the case of the c3158A>C polymorphism, patients harboring a C/C genotype had a lower OS than patients with a A/A or A/C genotypes (P = 0.047). We identified germline variants in LRIG1 gene predicting clinical outcome in patients with mCRC receiving first-line oxaliplatin/5-fluorouracil chemotherapy.

P17-06

Proantocyanidins in cytoprotection of antitumor drugs-treated cells

Katanic J (1), Stankov K (1), Srdjenovic B (2), Pavlovic N (2), Bogdanovic V (3), Bogdanovic G (3)

(1) Medical Faculty Novi Sad, Institute of Biochemistry, Novi Sad, Serbia
(2) Medical Faculty Novi Sad, Institute of Pharmacology, Novi Sad, Serbia
(3) Oncology Institute of Vojvodina, Experimental Laboratory, Sremska Kamenica, Serbia

Corresponding author: katanicj@open.telekom.rs

Introduction: Antitumor drugs Doxorubicin (DOX) and Mitomycin C (MMC) are broadly used antitumor drugs, but their clinical use is significantly limited due to their systemic toxicity. The main cause of their toxic effects is the oxidative stress as a consequence of ROS (reactive oxygen species) gener-

ation during the DOX and MMC metabolism. The main aim of our study was to investigate the efficacy of different antioxidative agents in oxidative stress prevention and protection from DOX and MMC effects, in normal (CHO – Chinese hamster ovary) and malignant cells (K562 – human erythroleukemia cells).

Materials and methods: The antioxidative agents used in our study were the glutathione precursor N-acetylcystein (NAC) and proantocyanidins (PAC), natural antioxidative compounds derived from the plant polyphenols. According to the aim of this study, the parameters of oxidant status, activity of antioxidant enzymes glutathione-S-transferase and glutathione reductase were tested in human erythroleukemia (K562) and Chinese hamster ovary (CHO) cells lines. Both cell lines were pretreated with potentially protective agents (NAC and PAC) 30 minutes before DOX and MMC, as prooxidant agents. Cell supernatants were prepared after 24 hours and GST and GR were determined, as parameters of oxidant status.

Results and conclusions: Results of our study suggest that proantocyanidins and N-acetylcysteine exert the antioxidative activity and therefore also the potentially protective activity to the effects of DOX and MMC. Comparative analysis of activities of antioxidant enzymes GST and GR in healthy (CHO) and malignant (K562) cells lines shows that none of those antioxidant agents expresses selective activity to non-malignant, CHO cells.

P17-07

YKL-40, IL-1β and TNF-α in malignant glioma

Kazakova M

Medical University - Plovdiv, Medical Biology, Plovdiv, Bulgaria

Corresponding author: kazakova25@abv.bg

Background: Glioblastoma multiforme (GBM) is the most aggressive brain tumour. YKL-40 is a glycoprotein that has been associated with glioma grade and a poorer clinical outcome. The aim of the investigation was to analyze tissue and serum YKL-40 levels in glial tumours in comparison with pro-inflammatory cytokines.

Materials and methods: Serum YKL-40, IL-1β and TNF-α levels were measured in 7 patients with GBM, 14 patients with lower-grade glioma (grades II and III) and 40 healthy controls, by ELISA method. An immunohistochemical analysis of YKL-expression was performed.

Results and conclusions: Feeble reactivity was determined in single glial cells in the normal brain. In astrocytoma the glycoprotein was present in numerous malignant cells. The most intensive reactivity was detected in GBM. Our study revealed a significant difference in YKL-40 serum concentrations between healthy subjects and lower-grade glioma, as well as between astrocytoma and GBM. The level of the glycoprotein in tumors was twofold higher than in controls ($P < 0.01$). We found an association between serum YKL-40 values and tissue YKL-40 expression. Enhanced IL-1β concentrations in glioma patients were detected. The secretion of TNF-α showed a similar pattern, but the increase towards the controls was lower compared to IL-1β. In conclusion, YKL-40 correlates positively with pathological tumor grades and might serve as a novel biomarker in malignant glioma. We revealed IL-1β and TNF-α – dependent (Th1) immune response in these malignant tumors.

Acknowledgments: The study is supported by grants NO–1/2009 and NO–1/2010 from Medical University – Plovdiv.

P17-08

Tumour markers in fluid analysis in the differential diagnosis of cystic lesions of the pancreas

Albuquerque M (1), Rego de Sousa M (2)

(1) Laboratório de Patologia Clínica Hospital CUF Infante Santo / Centro de Medicina Laboratorial Dr. Germano de Sousa, Clinical Pathology / Immunology, Lisbon, Portugal
(2) Centro de Medicina Laboratorial Dr. Germano de Sousa, Clinical Pathology / Immunology, Lisbon, Portugal

Corresponding author: rribeiro@cm-lab.com

Pancreatic cystic lesions include the most common pseudocysts and a variety of cystic tumours with different biologic and pathologic characteristics. With the increasing technological tools at our disposal, cystic neoplasm became more visible. Preoperative differential diagnosis of cystic lesions of the pancreas may be difficult because decision making through reliable clinical and, or imageological criteria is uncertain. In the last decade, the cystic fluid analysis using a panel of tumours markers and in some studies, serum pancreatic enzymes like amylase and lipase, combined with endoscopic ultrasonography fine needle aspiration, became an important tool in the pancreatic lesions workup and even in follow up. The authors propose to make the evaluation of the assay of tumour markers, CEA, CA 19.9, CA 125 in net drainage of cystic lesions and their application in the differential diagnosis of cystic pancreatic lesions. Drainage fluids were obtained in 195 patients with pancreatic cysts for a period of 6 years (2006 to 2012) with variable histological diagnoses: pseudocysts, mucinous cystic carcinomas, ductal carcinomas and cystadenomas. The aims of this study are to establish the cut off levels for our laboratory in the light of the study population as well as verify the correlation between the determinations of these biological markers and severity of histological lesions.

P17-09

Multiplex biochip arrays allow simultaneous assessment of serum markers for colon cancer screening

Bunger S (1), Fitzgerald S (2), Toner V (3), Buning J (4), von Eggeling F (5), Habermann J (1)

(1) University of Lubeck, Department of Surgery, Lubeck, Germany
(2) Randox Laboratories Limited, Research and Development, Crumlin, United Kingdom
(3) Randox Laboratories GmbH, Research and Development, Wulfrath, Germany
(4) University Hospital of Schleswig-Holstein, Internal Medicine I, Lubeck, Germany
(5) Jena University Hospital, Institute of Human Genetics, Jena, Germany

Corresponding author: Mariluz.Rodriquez@randox.com

Background: Colorectal cancer is the third most commonly diagnosed cancer in males and the second in females. Despite the implementation of screening programs such as colonoscopy, approximately 50% of patients are diagnosed at advanced tumor stages resulting in poor prognosis. Innovative, patient-friendly screening tools could aid in the early detection and allow curative treatment interventions.

Materials and methods: A nine target multiplex protein biochip was generated based on a thorough literature review. Diagnostic performance was evaluated using a training- and validation-set of a highly standardized, liquid nitrogen preserved serum collection of 317 samples comprising controls, adenomas, and colon cancers.

Results: Serum levels of CEA, IL-8, VEGF, S100A11, C3adesArg, CD26, MCSF and CRP showed significant differences between colon cancer cases and controls ($P < 0.05$). The largest areas under the receiver operating characteristics curve were observed for CEA(0.69), IL-8(0.68), and CRP(0.64). At threshold levels yielding specificities of 90%, the sensitivities for CEA, IL-8 and CRP were 26%, 22%, and 17%, respectively. Testing all possible combinations of these markers at the predefined threshold levels, CEA + IL-8 reached a sensitivity of 37%

at 83% specificity and CEA + CRP obtained a sensitivity of 35% at 81% specificity for detecting colon carcinomas.

Conclusions: Multiplex biochip array technology offers an innovative and patient-friendly approach to colorectal cancer screening. The diagnostic value of identifying further serum biomarkers and the potential advantage of combining biochip analysis with fecal occult blood has the potential to improve the performance of colorectal cancer screening and warrants further investigation.

P17-10

Identification of novel cancer biomarkers using the Randox-QuantiPlasm69 monoclonal antibody chip

Antal-Szalmas P (1), Lazar J (2), McGregor S (3), Muller M (4), Kurucz I (2), Kappelmayer J (1)

(1) Deptartment of Laboratory Medicine, University of Debrecen MHSC, Debrecen, Hungary
(2) Biosystems International Ltd., Debrecen, Hungary
(3) Randox Laboratories Ltd., Crumlin, United Kingdom
(4) Adware Research Ltd., Balatonfüred, Hungary

Corresponding author: alumni@med.unideb.hu

Background: Recently a novel monoclonal antibody based protein chip – QuantiPlasm69 (QP69) – has been introduced by Randox Laboratories. This system uses 69 monoclonal antibodies (mAbs) – developed by BioSystems International – that are immobilized on 9x9 mm ceramic chips. The QP69 assay can recognize concentration changes of several human plasma proteins simultaneously and in this way can identify novel plasma markers in a wide variety of diseases.

Materials and methods: Plasma samples and clinical data of 150-150 patients with prostate and lung cancer and 300 healthy controls were collected. Individual and pooled samples of the patients and controls were evaluated by the QP69 system. The plasma pools were created from the individual samples based on clinical, histopathological and laboratory data. Other biochemical parameters and the classical tumormarkers were also measured. To find the most predictive parameters principal component, binary logistic regression and ROC analysis was performed beside the classical statistics.

Results: A set of mAbs (three antibodies) present on the QP69 chip were able to discriminate between healthy controls and lung cancer patients, and a different set of mAbs (three antibodies) were able to distinguish samples of healthy controls from those of prostate cancer patients. These differences were independent of age and smoking habits. Combination of these antibodies in themselves or with classical tumormarkers could further improve their efficacy.

Conclusions: The QP69 kit can be an effective tool in biomarkers' search and discovery. This work was supported by the National Office for Research and Technology of Hungary (TECH-09-A1-2009-0113; mAB-CHIC).

P18 - Oncology - Tumor marker 2

P18-01

Evaluation of tumor marker HE4 assay on the Elecsys 2010 analyzer

Ćorić J

Clinical Center of Sarajevo University, Department of Clinical Chemisty, Sarajevo, Bosnia and Herzegovina

Corresponding author: coricjozo@hotmail.com

Background: Whey-acidic protein human epididymis protein4 (HE4), a new promising biomarker for epithelial ovarian cancer (EOC). The measured HE4 value of patients sample can depending on the testing procedure use.

Methods: We evaluated a HE4 method on Elecsys 2010 analyzer. The method for quantitative determination of HE4 is direct, competitive electrochemiluminescence immunoassay. For quality control we use Elecsys PreciControl HE4 1 and 2. HE 4 was measure on sera obtained from 96 women (40 healthy and 56 with epithelial ovarian cancer).

Results: The Roche HE 4 assays showed a good linearity (r = 0.99) and precision (intrassay and total CV < 5%). The median HE4 serum concentrations was significantly higher among EOC patients than healthy females (P < 0.05). As a single marker, HE4 had a sensitivity of 78.4 % with a specificity of 95 %.

Conclusions: The presented results of the analytical evaluation methods for the determination of HE 4 on the Elecsys 2010 analyzer showed an acceptable accuracy and precision.

P18-02

CYFRA 21-1 new tumor marker in Abbott iArchitect family

Županić D, Crnokrak S, Mujagić R, Honović L

Pula General Hospital, Department of Laboratory Diagnostics, Pula, Croatia

Corresponding author: danijela.zupanic@pu.t-com.hr

Introduction: CYFRA 21-1 is a tumor marker that measures fragments of Cytokeratins 19 using two monoclonal antibodies KS 19-1 and BM 19-21, but its main role is to monitor the course and success of the therapy of NSCLC.

Objective: Our goal was to investigate the acceptability of Abbott reagents for use in daily routine on immunochemical analyzer Architect i2000SR to ensure traceability of our results. Validation of immunoassays was performed using CLSI / NCCLS procedure EP 15-A2.

Materials and methods: The following parameters were assessed: Precision (random error) in which we determined repeatability, interprecision and overall laboratory precision by determination of a small group (N = 30) and systematic error (deviation from expected values).

Results:

- Control Level 1: 5 ng / mL (3.5 to 6.5) (Repeatability x = 5.23, Sr = 0.22, KV% = 4.2%)
- (Interprecision x = 5.23, Sb = 0.123, KV% = 2.4%)
- (Overall laboratory precision SL = 0.218, KV% = 4.2%)
- (Systematic error is 0.23 (4.6%))
- Control Level 2: 35 ng / mL (24.5 to 45.5) (Repeatability x = 34.92 ng / ml, Sr = 0.44, KV% = 1.3%)
- (Interprecision x = 34.92, Sb = 0.524, KV% = 1.5%)
- (Overall laboratory precision SL = 0.635, KV% = 1.8%)
- (Bias -0.08 (-0.23%))

Conclusion: Based on the results of this study, we can conclude that the tested reagent CYFRA 21-1 Abbott is acceptable and we recommend its use on automated immunochemical analyzer iArchitect Abbott.

P18-03

Analytical performance of tumor markers on UniCel DxI 600 using Sigma metrics

Unic A, Derek L, Marijancevic D, Serdar T, Romic Z

University Hospital Dubrava, Clinical Department for laboratory diagnostics, Zagreb, Croatia

Corresponding author: aunic@kbd.hr

Background: The Sigma concept of a tolerance limit provides guidance for defining intended medical use in the form of an allowable total error. Performance is characterized on a sigma scale. In terms of Sigma, if a method has a value less than three is considered to be unreliable and should not be used in routine laboratory practice. The aim of this study was to assess the performance of automated immunodiagnostic chemiluminescence system UniCel DxI 600 (Beckman-Coulter, Tokyo, Japan) for qualitative detection of tumor markers.

Materials and methods: For CA19-9, CA15-3, CA125, and PSA method validation Lypochek Tumor Marker (Bio-Rad Laboratories, Marnes-la-Coquette, France; Control lot 54530- Level 1 and 3) control samples were obtained. All immunoassays were preformed on the UniCel DxI 600 analyzer

according to the manufacturer instructions. Control samples were tested in triplicate every day during the 5 days according CLSI/NCLLS protocol EP15-A2. Bias was calculated using method comparison. Sigma was calculated using Ricos quality requirements.

Results: Calculated sigma metrics for CA15-3 were 2.5 and 1.4; for CA125 6.5 and 5.9; for CA19-9 6.4 and 9.2; and for PSA-1.0 and 2.2.

Conclusion: Analytical performance for CA125 and CA19-9 is world class. On the other hand, CA15-3 and PSA analytical performance is poor or even unacceptable. Using graphic tool it becomes apparent that bias is the main problem with CA15-3 and PSA performance. With lower bias CA15-3 assay performance might even reach Six Sigma zone. Therefore, it is necessary to use the same method for individual patient. In that case, even these two methods became of excellent quality.

P18-04

Prognostic value of Cyfra 21-1, CEA and NSE in patients with NSCLC

Musliu A

Faculty of Natural Science, Department of Biology, Tirana, Albania

Corresponding author: milazimshabani@hotmail.com

Background: The aims of this study were to evaluate prognostic effects, sensitivity and specificity of Cyfra 21-1 in detecting non-small cell lung cancer.

Subjects and methods: The study included 118 randomly selected patients with NSCLC compared with control group of 30 patients with nonmalignant pulmonary disease. Histology tumor diagnosis was based on biopsy specimens obtained at bronchoscopy, lymphode biopsy or thoracotomy. Tumor markers (Cyfra 21-1,CEA and NSE) were assayed in Immunological Abbot's Axsym System analyzers. Chemotherapy included cisplatin and carbocisplatin were aplicated in doses (80-100 mg/m^2 day 1.3 wk cycle and 200-300 mg/m^2 day 1.3 wk cycle for six cycles).

Results: The level of tumor markers was determined in both population in different stage of age before and after chemotherapy. Median level of Cyfra 21-1 at diagnosis was 36.2 ng/mL with range 2.8-215.0 and decreased significantly after second cycles of chemotherapy 24.3 ng/mL (range 3.4-145.0; $P < 0.01$). Same results we obtained in the NSE concentration (23.4 µg/mL before and 14.2 µg/mL after therapy, but CEA shows not significantly changes. Cyfra 21-1 were elevated in 22.3%, NSE in 10.8% and CEA in 16.5% of patients respectively.

Conclusion: Cyfra 21-1 reflects the extent of the disease and has an independent prognostic role along with performance status and disease stage in NSCLC.Combining Cyfra 21-1, NSE and CEA correlated with prognosis in a significant and independent manner.

P18-05

Computational screening for non-small cell lung carcinoma biomarker candidates

Dundovic S (1), Debeljak Z (2), Seric V (2)

(1) General Hospital "Nasice", Department of Medical Biochemistry, Nasice, Croatia
(2) Clinical Hospital Center "Osijek", Department of Clinical Laboratory Diagnostics, Osijek, Croatia

Corresponding author: zeljko.debeljak@gmail.com

Introduction: Comparison of gene expressions obtained on control and patient samples based on computational methods allows selection of relevant genes and their protein products which may serve as biomarker candidates. For the purpose of non-small cell lung carcinoma biomarker screening a multivariate classification method known as k-nearest neighbors has been selected in this study.

Materials and methods: The study was conducted on a publicly available set of gene expressions

(Showe MK et al. Cancer Res 2009;69:9202-10) which consists of 291 gene expression profiles (137 non-small cell lung carcinoma patient samples and 91 control samples), each containing 15 227 gene expressions. k-nearest neighbors method was applied to all pairs of gene expressions. t-test with Benjamini-Hochberg correction for multiple testing has been used as a univariate filter.

Results: Application of k-nearest neighbors resulted in the test set accuracy of 81.6-86.8% which corresponds to 1000 top ranking gene pairs. Less than a half of highly ranked genes were statistically significant according to the corrected t-test. The majority of the top ranking genes are responsible for the processing of gene information and for the regulation of signaling pathways.

Conclusion: k-nearest neighbors method provides insight into the relevance of single genes and gene interactions for differentiation of patient and control samples. Low correspondence between univariate results and the results of k-nearest neighbors underlines importance of the latter. This might influence the quality of candidates for non-small cell lung carcinoma biomarkers and corresponding diagnostic approaches which laboratory evaluation is underway.

P18-06

Prognostic and predictive significance of ERβ1 in primary breast cancer

Jelisavac Ćosić S (1), Jakić-Razumović J (2), Sirotković-Skerlev M (3)

(1) University Hospital Centre Zagreb, Department of Oncology and Radiotherapy, Zagreb, Croatia
(2) University Hospital Centre Zagreb, Zagreb University Medical School, Department of Pathology and Cytology, Zagreb, Croatia
(3) Zagreb University Medical School, Department of Pathophysiology, Zagreb, Croatia

Corresponding author: jelisavaccosic.sanda@gmail.com

Background: Adjuvant endocrine therapy is effective treatment in breast cancer. Patients receive endocrine treatment according to steroid receptor positivity (ERa and PgR). Estrogen receptor β (ERβ) is a second estrogen receptor and its role in endocrine treatment is not fully elucidated. The aim of this research was to determine prognostic and predictive value of isoform EBβ1 in breast cancer.

Materials and methods: In the study were included 150 consecutive primary breast cancer cases operated at University hospital Zagreb (year 2002-2003). Overall and disease free survival were recorded until January 1st, 2011. Immunohistochemistry was used for EBβ1 determination (anti-ERβ1 clone PPG5/10, Dako, Denmark).

Results: ERβ1 and ERα expressions were not correlated (P = 0.178, r = 0.123). There was no association of ERβ1 with age, menopausal status, lymph node status, tumor size, histological grade, histological type, nuclear grade, Nottingham prognostic index, proliferation index Ki67 expression, steroid receptor status and HER-2/neu status. In univariate Cox-regression analysis, ERβ1 was associated with overall survival (P = 0.026; HR = 0.46; 95%CI 0.24-0.92) but there was no association with disease free survival (P = 0.054; HR = 0.51; 95%CI 0.25-1.04). Kaplan-Meyer survival curve analysis confirmed prognostic significance of ERβ1 for overall survival. In the subgroup of patients that received endocrine treatment (N = 93), there was no statistical difference in survival between patients with high and those with low ERβ1 [overall survival (P = 0.27; HR = 1.73; 95%CI 0.65-4.7), disease free survival (P = 0.08; HR = 2.25; 95%CI 0.91-4.94)].

Conclusions: Cancer tissue expression of ERβ1 is a prognostic, but not a predictive marker in primary breast cancer.

P18-07

Comparative study of two chemiluminescent methods for determining free PSA

Fernandez Leivas A, Garcia Moreira V, Llorente Torres A, Beridze N, Garcia Alonso S, Fernandez Rodriguez E

Hospital de Cabueñes, Servicio de Analisis Clinicos, Gijon, Spain

Corresponding author: anafleivas@hotmail.com

Background: The % free PSA (fPSA) is, on average, lower in prostate cancer than the benign prostatic hyperplasia and has been commonly used as an aid in the diagnosis of prostate cancer when PSA is between 4-10 ng/mL.

Materials and methods: In our study, fPSA were analyzed in 50 serum samples of patients with total PSA between 4-10 ng/mL. The results obtained using the methods ADVIA Centaur® fPSA test (Siemens) and fPSA Immulite2000 (Siemens), were compared in order to ensure transferability of results between both methods. Total PSA was measured by ADVIA Centaur® method. Analysis of data was performed using the MedCalc®, using Passing-Bablok nonparametric regression test, Pearson´s correlation and agreement between methods was evaluated using the Kappa statistics, according to the following groups: positive > 15% and negative < 15% fPSA.

Results: The following values of fPSA were obtained (range, median) 0.39-2.72, 1.191 (95% CI: 1.037 to 1.346) and 0.27-2.46, 1.135 (95% CI: 0.985 to 1.285) for Immulite2000 and ADVIA Centaur® respectively, yielding a correlation coefficient of 0.9519 between both methods ($P < 0.001$). The Passing-Bablok regression equation obtained had a Slope = 0.9583 (95% CI 0.8729 to 1.0596) and Intercept = 0.0196 (95% CI: -0.0837 to 0.1084). The concordance of % freePSA is good, yielding a Kappa index of 0.797 (95%CI: 0.628-0.966).

Conclusions: Both methods of fPSA show a good correlation ($0.9 <= R <1$) and concordance. Results between methods are transferable.

P18-08

Soluble urokinase plasminogen activator receptor (suPAR) in breast cancer

Tarapacz J, Rychlik U, Wójcik E, Stasik Z, Kulpa J

Center of Oncology – Maria Sklodowska-Curie Memorial Institute, Cracow Division, Poland, Clinical Biochemistry, Kraków, Poland

Corresponding author: z5tarapa@cyfronet.pl

Introduction: The urokinase plasminogen activator system plays an important role in many processes involved with cancer invasion and metastasis. The elevated levels of uPAR as well as uPA and PAI-1 are associated with poor prognosis in cancer patients. Recently, similar suggestions are brought forward to soluble form of this receptor – suPAR. The aim of study was the evaluation of relationship between suPAR and selected biochemical and clinical parameters in breast cancer patients.

Material and methods: The study of suPAR, CA 15-3, CEA, CRP was performed in the group of 146 breast cancer patients before surgical treatment and in the reference group of 43 healthy women.

Results: In breast cancer patients in comparison with the reference group there were found significantly higher levels of suPAR, CA 15-3, CEA and CRP. Significantly higher levels of CA 15-3 had patients in more advanced stages of disease, with tumor greater than 2 cm, and CRP levels > 3 mg/L. There were no significant differences between analyzed biochemical factors between groups selected in respect to: lymph node status, histological grade, percentage of S-phase cells, steroids receptor status. In the group of breast cancer patients with HER2 overexpression significantly lower suPAR and significantly higher CA 15-3 levels and the percentage of S-phase cells there were observed.

Conclusions: In breast cancer patients suPAR level seems to be associated with tendency to inflammation. The reciprocal relationship between HER2 status and suPAR levels may indicate potential role of this receptor in prognosis of some breast cancer patients.

P18-09

BRAF: potential prognostic marker in colorectal cancer

Rako I (1), Jakić-Razumović J (2), Sabol I (3), Sertić J (1), Pleština S (4)

(1) University Hospital Center Zagreb, Department of Laboratory Diagnostics, Zagreb, Croatia
(2) University Hospital Center Zagreb , Department of Pathology and Cytology, Zagreb, Croatia
(3) Institute Rudjer Boskovic Zagreb, Laboratory of Molecular Virology and Bacteriology, Zagreb, Croatia
(4) University Hospital Center Zagreb, Department of Oncology, Zagreb, Croatia

Corresponding author: irako@kbc-zagreb.hr

Introduction: Incidence and mortality of colorectal cancer (CRC) are on constant increase and represent one of the major health problems in Croatia. Activation of BRAF oncogene is implicated in colorectal carcinogenesis. BRAF protein is a serin/threonine kinase, component of a conserved signaling pathway that regulates cellular responses to exstracellular signals. In human cancer, it is commonly activated by hotspot mutation of the BRAF gene which leads to a single aminoacid substitution p.V600E. The aim was to determine the incidence of BRAF gene mutations in CRC patients in Croatia and to assess whether they are linked with clinicopathological features of poor prognosis.

Materials and methods: Sections from 113 formalin-fixed paraffin-embedded tumor samples were evaluated for the BRAF mutation using LightCycler PCR with allele-specific fluorescent probe melting curve analysis. Obtained results were confirmed by sequencing method.

Results: Our results show that BRAF gene mutation p.V600E was detected in 8.8% (10/113) CRC samples. Statistical analysis revealed a significant association between the BRAF mutation and Dukes'stage (P = 0.04) where all mutations were found in tumors classified as Dukes'C. Incidence of mutation was higher in males, patients older than 60 years, tumors bigger than 5 cm, tumors with angioinvasion and poor differentiated tumors, but no significant association was found. All BRAF gene mutations were detected in colon cancers.

Conclusions: The incidence of BRAF gene mutation in CRC patients in Croatia is within commonly accepted limits. Higher incidence in tumors with poor prognostic markers shows that BRAF gene mutation play a role in the progression of CRC.

P18-10

Assessment of serum CA 15-3 and CEA concentrations in patients with breast cancer

Tuteska J (1), Stojkovski V (2), Arapceska M (3), Lozanovski Z (1), Risteska K (1)

(1) University "St. Kliment Ohridski", Medical Nursing College, Depatment of Biochemistry, Bitola, Macedonia, TFYR
(2) University "Ss. Cyril and Methodius", Faculty of Veterinary Medicine, Depatment of Biochemistry, Skopje, Macedonia, TFYR
(3) University "St. Kliment Ohridski", Faculty of Biotechnical Sciences, Department of Natural Sciences, Bitola, Macedonia, TFYR

Corresponding author: jtuteska@yahoo.com

Background: Breast cancer is the most common malignancy in women. The aim of this study was assessment of serum CA 15-3 and CEA concentrations in patients with breast cancer and monitoring of their status by these tumor markers.

Material and methods: Serum samples were acquired from female patients with breast cancer (N = 224) grouped according pTNM staging. Control samples (N = 44) were collected from healthy females. Concentrations of tumor markers were measured by the immunochemical analyzer Vitros ECI with enhanced chemiluminiscence.

Results: According obtained results concentrations of CA 15-3 in patients in grade IIIb (102.50 ± 10.61 U/mL) and grade IV (134.50 ± 179.74 U/mL) were 86.67% and 89.84% significantly higher ($P < 0.01$, $P < 0.05$ respectively) than in healthy women (13.66 ± 8.55 U/mL). Concentration of CEA in patients in grade IV (19.3 ± 17.4 ng/mL) was 92.64% higher ($P < 0.01$) compared with healthy women (1.42 ± 1.02 ng/mL). Three months after surgery in patients was noted increase of CA 15-3 values,

which after six months were 71.14% higher, despite CEA values that were in referent range.

Conclusions: Because of low sensitivity tumor markers CA 15-3 and CEA are more suitable for monitoring of state of patients after surgery than for early screening or diagnosis of breast cancer.

P19 - Pregnancy

P19-01

Results of first trimester screening for Down's, Edward's and Patau's syn using SsdwLab5 software

Spasić Obradović G (1), Ille K (1), Mitrović J (2), Milatović Jezdić V (3)

(1) Zvezdara University Medical Centre, Laboratory of Clinical Biochemistry, Belgrade, Serbia
(2) University Childrens Hospital, Laboratory of Clinical Biochemistry, Belgrade, Serbia
(3) Primaray Health Centre Savski Venac, Laboratory of Clinical Biochemystry, Belgrade, Serbia

Corresponding author: spasicgordana@yahoo.com

Background: First trimester screening is indispensable in early identification of Downs, Edwards and Pataus syndrome in fetuses. We performed combined protocol which included: maternal biochemistry (free beta-hCG and PAPP-A) and sonografic determination of nuchal translucency (NT) during year 2011. Screening was performed between 11+0 and 13+6 weeks of pregnancy. Risk calculation was performed using the software SsdwLab version 5.0. This software makes use of an algorithm described by Palomaki and is based on the mathematical calculation using Gaussian multivariate distribution. Risk analysis is based on maternal age, NT as well as on the results of biochemical parameters, corrected by different factors like e.g. maternal weight, smoking and ethnic background of the pregnant woman.

Materials and methods: Free beta-hCG and PAPP-A were performed by electrochemiluminescence immunoassay on COBAS E 411 immunoassay analyzer.

Results and conclusions: Total of 1459 samples from clinical routine with known outcome were examined in year 2011. 42 out of the 1459 samples were from pregnancies with confirmed Down's syndrome. 8 out of the 1459 samples were positive for Edward's or Patau's syndrome. Cut-offs for Trisomy 21 and 18-13 were 1/250.

P19-02

The role of antenatal screening and amniocentesis on the Down's syndrome diagnosis – our experiences

Hoxha-Muhaxhiri M (1), Muhaxhiri F (2)

(1) Medical Institute FATI IM, Clinical Laboratory, Prishtinë, Kosovo
(2) Medical Institute FATI IM, Gyn/Obs, Prishtine, Kosovo

Corresponding author: drmimozahoxha@hotmail.com

Description: Antenatal screening identifies high risk pregnancies for Down's Sy. The screen includes the risk assessment based on data processing of the maternal serum PAPP-A and Fß-hCG and the NT. It is performed between 11w0d-13w6d of gestation. According to the risk assessment women are distinguished into the high risk group (> 1/250) screen positive, and low risk group (< 1/250) screen negative.

Aim: Assessment of correlation between the screening high risk pregnancies and the amniocentesis in our center.

Material and methods: Study included 472 pregnant women tested between 02/2011-04/2012, 22-42 years, gestation 11+0 to 13+6 weeks. The testing of the maternal biomarkers has been performed on Roche Elecsys2010. Measurement of the NT has been performed by Toshiba-XarioXG ultrasound. The risk has been calculated on FMF's software, where the woman age, CRL, parity, smoking, BMI and ethnic origin has been evaluated too. Amniocentesis has been performed between 17w 1d-20w 6d weeks of gestation on ultrasound guided free hand technique using the 22G needle.

Results: Among 472 tested, with the high risk on Trisomy21 (> 1/250) resulted 26 (5.5%) which then have been recommended for amniocentesis. 23 of 26 woman have undergone the amniocentesis whilst 3 refused the procedure. Among 23 performed amniocentesis 2 of 23 (7.69%) or (4.2‰) cases of the total number tested, resulted with Trisomy21 and have been referred for pregnancy termination.

Conclusions: Antenatal screening is a non invasive test, able to be realized in the early gestation, technically easy to be performed and cost effective.

P19-03

Standard and new laboratory markers and their usefulness in prediction of preeclampsia

Štefanović M (1), Delić R (2), Krivec Š (3)

(1) Sestre milosrdnice University Hospital, Clinical Institute of Chemistry, Zagreb, Croatia
(2) General Hospital Celje, Department of Obstetrics and Gynecology, Celje, Slovenia
(3) General Hospital Celje, Department of Laboratory Medicine, Celje, Slovenia

Corresponding author: mstefan6@gmail.com

Background: Syndrome preeclampsia occurs in 5 to 8% of pregnancies and is a leading cause of maternal and neonatal morbidity and mortality. There are no clinically available tests that perform well in predicting development of preeclampsia at the moment. The aim of this study was to investigate the predictive potential of standard laboratory parameters and to evaluate new angiogenic markers at third trimester of pregnancy in prediction of preeclampsia.

Patients and methods: First, retrospective designed study included 113 patients with preeclampsia and a control group of 95 uncomplicated pregnancies. It evaluates erythrocytes, leukocytes, thrombocytes, hemoglobin, hematocrit, AST, ALT, GGT, alkaline phosphatase, total bilirubin, urea, creatinine, uric acid, body mass index, parity, age, and blood type in prediction of preeclampsia based on multivariate logistic regression model. In the second, prospective study data from 34 patients with preeclampsia and 35 patients with uncomplicated pregnancies were evaluated. New angiogenic markers, PlGF, s-Flt-1 were evaluated together with standard laboratory parameters, the same way as in the first study.

Results: When parameters such as uric acid and urea were included into logistic regression model, we correctly classified 79.6% patients. Additional parameters (thrombocytes, hematocrit, aspartate aminotransferase and leukocytes) raised correct classification to 83.8% patients and employing PlGF, sFlt-1 with other parameters, percentage of correctly classified patients reached 94,3%.

Conclusion: Standard laboratory parameters, when used as laboratory test panel have significant prognostic value in the prediction of preeclampsia, but new angiogenic markers showed to be superior and can sucesfully predict occurence of preeclampsia at lower gestational age.

P19-04

Comparison of the „SsdwLab 5.0.9“ and „Fetal Medicine Foundation“ Down syndrom screening softwares

Radeljak A (1), Podobnik Brlecic P (2), Ožvald I (1), Gebauer Vukovic B (2), Podobnik M (2), Stojanovic N (1)

(1) University Hospital Merkur, Institute of Clinical Chemistry and Laboratory Medicine, Zagreb, Croatia
(2) Clinic „Podobnik", Clinic „Podobnik", Zagreb, Croatia

Corresponding author: radeljak.andrea@gmail.com

Background: First trimester screening by a combination of maternal age, fetal nuchal translucency and maternal serum free-β-hCG and PAPP-A can indentify about 85-90% of fetuses with trisomy 21 and other mayor aneuploidies. The aim of this study was to compare risk calculations perfomed using two different softwares.

Materials and methods: PAPP-A and free-β-hCG concentrations were measured by electrochemilu-

minescent immunoassay (ECL) on Roche Elecsys analyzer. The obtained results were within the target values of internal and external quality assurance programme (UKNEQAS scheme for First Trimester Downs Syndrome Screening). Nuchal translucency was measured by sonographers with FMF Certificate of Competence. Risk calculations were performed using „SsdwLab 5.0.9"(Roche, certified by FMF) and „Fetal Medicine Foundation" softwares.

Results: Patients (N = 247) were devided into high, intermediate and low risk grupe. The obtained distributions per group were: high risk 18 (7.29%), intermediate risk 36 (14.57%) and low risk 193 (78.14%) patients using SsdwLab 5.0.9 software vs. high risk 12 (4.86%), intermediate risk 26 (10.53%) and low risk 209 (84.61%) patients using FMF software. 18 patients were classified as high risk by SsdwLab 5.0.9 vs. 10 (55.6%) as high risk and 8 (44.4%) as intermediate risk using FMF software. Only one fetus from the high risk group (by both softwares) was trisomy 21 positive, which was confirmed by amniocentesis.

Conclusions: Comparing risk calculations performed using „SsdwLab 5.0.9" and „Fetal Medicine Foundation" softwares, we concluded that SsdwLab 5.0.9 calculates higher risks for the same input parameters. Further research will be aimed at discovering the causes of observed differences.

P19-05

What do the triple test results mean?

Popovic D (1), Vujovic S (2), Stanisic N (3)

(1) Special Hospital "Codra", Podgorica, Montenegro and Faculty of Natural Sciences, University of Montenegro, Podgorica, Montenegro
(2) Faculty of Natural Science, University of Montenegro, Podgorica, Montenegro
(3) Special Hospital "Codra", Department for Gynecology, Podgorica

Corresponding author: midpopovic@t-com.me

Introduction: The triple test is a screening test and not a diagnostic test. This test only notes that a mother is at a possible risk of carrying a baby with a genetic disorder The triple screening test is performed between the 14th and 22nd week of pregnancy. This test is recommended for women who have a family history of birth defects, are 35 years or older.

Materials and methods: These results represent three years study of screening for genetic disorders (trisomy 21, trisomy 18 or another tip of chromosome abnormality). Triple screening test is a combination of maternal age, weight, ethnicity, gestation of pregnancy, biparietal diametar (BPD) and serum total HCG, AFP and unconjugated estriol. We analysed 168 serum samples from pregnant women at the second trimester by Immulite 1000 (Siemens, risk assessment software PRISCA 4). The distribution of estimated risks for trisomy 21 was determined and sensitivity and false-positive rate for a risk cut-off 1 in 250 were calculated.

Results: The average maternal age was 30 (range 16-44) years and in 26 (15%) the age was 35 years or more, the average gestation at screening was 15.9 (14-22) weeks and average of BPD was 35 (range 29-56) mm. The estimated risk for trisomy 21 based on mentioned parameters was 1 in 250 or greater in 3.0% (4 of 168) of pregnancies.

Conclusion: Our result of screening for chromosome abnormality by measuring of fetal BPD and maternal biochemical parameters are similar to those reported in literature.

P19-06

Analysis of biochemical parametars in the perinatal screening

Vujovic S (1), Popovic D (2), Ajanovic E (1), Jovanovic M (3)

(1) Faculty of Science, University of Montenegro, Biology, Podgorica, Montenegro
(2) Special Hospital "Codra", Biochemistry, Podgorica, Montenegro
(3) Primary Health Care Centre, Gynecology, Podgorica, Montenegro

Corresponding author: slavica.vujovic@t-com.me

Introduction: The pregnancy is specific condition of the body followed by a number of changes and different physiological states, so that a phenomenons that are the subject of this study is gestational diabetes and pregnancy induced hypertension. Aim of this study was to determine whether the deviations from the reference values of β-human-chorion gonadothropin and pregnancy-associated plasma protein-A are correlated with certain disorders of pregnancy such as pregnancy-induced hypertension, or gestational diabetes, and whether these parameters can be used in the prediction these complications in pregnancy.

Materials and methods: In these study are analised 50 pregnant women. In the control group were 20 physiologically healthy pregnant women; in the study group of fifteen pregnant women (50%) had established PIH, and the other fifteen (50%) were diagnosed with gestational diabetes. Biochemical analysis was done by testing the imunofluoroscency (Wallac DELFIA Xpress). Results are expressed as multiples of median (MoM) specific gestational age.

Results: In these research found no discrepancy between the values of biochemical parameters in the study and control group. The value of βhCG and PAPP-A at physiologically normal pregnant women and pregnant women diagnosed with PIH and gestational diabetes had no significant deviation.

Conclusions: Based on analysis of data in the form of biochemical screening of pregnant women age and number of deliveries we could not see a significant shift in the diagnosis and prediction of when it comes to certain complications of pregnancy such as gestational diabetes and pregnancy induced hypertension.

P19-07

SNP's in LEP gene and LEPR gene are associated with recurrent spontaneous abortions (RSA)

Wagner J (1), Muller A (2), Škrlec I (1), Heffer M (1), Maver A (3), Peterlin B (3)

(1) Faculty of Medicine, Department of Medical Biology, Osijek, Croatia
(2) University Hospital Centre Osijek, Clinic for Gynecology and Obstetrics, Osijek, Croatia
(3) University Medical Center Ljubljana, Institute of Medical Genetics, Ljubljana, Slovenia

Corresponding author: jwagner@mefos.hr

Aim: Single nucleotide polymorphisms (SNP) in leptin (*LEP*) and leptin receptor (*LEPR*) genes were compared between a group of female patients with more than three recurrent spontaneous abortions (RSA) and women with two or more successful pregnancies (SP).

Materials and methods: In a cohort study 145 women with SP and 178 women with RSA were tested. Genotype of four SNP's in LEP (rs7799039, rs2122627, rs11761556, rs10244329) and four SNP's in LEPR (rs1137101, rs7516341, rs1186403, rs12062820) were determined using KASP SNP Genotyping system and ABI Prism 7000 SDS instrument. Statistical comparison was done using Chi–square statistic. The haplotype frequencies and haplotype–disease associations were estimated using haplo.stats package.

Results: The genotype frequencies did not deviate from HWE, except in the case of one *LEP* and two *LEPR* SNPs. In the case of rs7799039 P value for RSA and all examinees was 0.03. The recessive model (AG + GG/AA) revealed significant association between 2548A genotype and RSA (OR = 1.58). Also, two SNPs from intron region of LEPR

(rs7516341 and rs1186403) deviated from normal distribution. In dominant model (CC + TC/TT) of the first SNP allele C decreases risk of RSA (P = 0.034, OR = 0.61). The second SNP was significantly different for SP group (P = 0.008) where T allele is of limited protective effect in the recessive model of inheritance (Chi-square P = 0.082, OR = 0.51).

Conclusion: It is known that mother's BMI during pregnancy influence maternal and newborn health. *LEP* and *LEPR* are candidate genes for RSA and therefore their influence on mother's BMI during pregnancy and final outcome of pregnancy deserves further investigation.

P19-08

The effect of pomegranate seed oil on histological features of testis and sperm quality in male rats

Nikseresht M (1), Mahmoudi R (1), Sharifi A (2), Rahimipour Sisakht F (3), Sane F (4), Jafari M (5)

1) Yasuj University of Medical Sciences, Cellular and Molecular Research Center, Yasuj, Iran, Islamic Republic of
(2) Yasuj University of Medical Sciences, Microbiology, Yasuj, Iran, Islamic Republic of
(3) Jahrom Branch, Islamic Azad University, Biology, Jahrom, Iran, Islamic Republic of
(4) Kazerun Branch, Islamic Azad University, Biology, Kazerun, Iran, Islamic Republic of
(5) Yasuj University of Medical Sciences, Histology, Yasuj, Iran, Islamic Republic of

Corresponding author: nipooya@gmail.com

Background: Pomegranate fruit extracts have been commonly marketed as dietary supplements in recent years because its health benefits have been shown in many studies. Many investigators have shown that the pomegranate possesses antioxidant activity and may act as a free radical scavenger.

Materials and methods: POMo (pomegranate seed oil) was extracted by petroleum ether. Male Wistar rats were divided into four groups (six per group). One milliliter corn oil, 200, 500 and 1000 mg/kg body weight POMo were given daily for seven weeks by gavage to first- 4th groups, respectively. After this period of time, epididymal sperm were collected and the indexes of sperm quality were determined in all groups. The lipid peroxidation levels and the reduced glutathione contents of sperm were measured. The Diameter of seminiferous tubules (µm) and germinal cell layer thickness (µm) of testis were determined.

Results: A significant increase was found in the percentage of forward progressive sperm motility and a significant decrease was found in the percentage of sperms with slow movement in rats treated with different doses of POMo. The epididymal sperm concentration in rats that eat POMo was higher than control group. The biochemical investigation showed that POMo increased the level of reduced glutathione and decreased the level of lipid peroxidation in the sperm. The Diameter of seminiferous tubules (µm) and germinal cell layer thickness (µm) were higher in treatment rats.

Conclusion: This study shows potential effect of POMo on sperm quality and may use for increasing the fertilizing potency of sperm.

P20 - Renal replacement

P20-01

Monitoring serial creatinine results in kidney transplant patients using the StatSensor POCT device

Cobbaert C (1), Romijn F (1), van Lint C (2), Schenk P (1), van der Boog P (2)

(1) Leiden University Medical Center, Department of Clinical Chemistry, Leiden, Netherlands
(2) Leiden University Medical Center, Department of Nephrology, Leiden, Netherlands

Corresponding author: c.m.cobbaert@lumc.nl

Background: Reference Change Values (RCVs) are helpful to interpret changes in serial diagnostic results. After renal transplantation creatinine is monitored frequently to detect rejection. To judge the usefulness of a device for home monitoring of creatinine, StatSensor POCT device (Nova Biomedical)

and central laboratory creatinine RCVs were determined, using a split sample comparison approach.

Materials and methods: Finger pricks were taken from 38 stable post-transplant patients (creatinine 50-450 µmol/L), and whole blood StatSensor measurements were performed. In addition, venous blood was sampled in lithium-heparin tubes and in serum separation tubes. Heparinised whole blood was used for replicate creatinine measurements on the StatSensor, and serum creatinine was determined using an IDMS-traceable enzymatic method on the Roche Modular P800. RCVs were calculated from: 2.8 x (CVa2 + CVb2)1/2 ($P < 0.05$, CVb = 5.3%).

Results: Mean creatinine levels on the StatSensor were 161 ± 86 and 154 ± 81 µmol/L in finger prick and venous whole blood, respectively. Serum creatinine levels on the Modular were 172 ± 82 µmol/L. Mean overall StatSensor CVa was 10.4% for finger prick and 5.2-6.6% for venous blood. Overall CVa for laboratory serum creatinine was < 1.5%. StatSensor RCVs were 35% and 23% for finger prick and venous whole blood, respectively, compared to 15.5% for the laboratory method.

Conclusions: Our data illustrate that RCVs are highly affected by overall CVa of POCT devices. Insufficient precision of the StatSensor causes a 2.3-fold increase in RCV as compared to the central laboratory method. A quality mark for POCT devices is recommended to guarantee desirable analytical performance.

P20-02

Falsely decreased ionized calcium in a dialysis patient

Lukić I (1), Lapić I (1), Fressl Juroš G (1), Brunetta Gavranić B (2), Rogić D (1)

(1) Clinical Hospital Center Zagreb, Clinical Institute for Laboratory Diagnostics, Zagreb, Croatia
(2) Clinical Hospital Center Zagreb, Department for Nephrology, Arterial Hypertension and Dialysis, Zagreb, Croatia

Corresponding author: lukic_iva@hotmail.com

Background: Administration of an anticoagulant is required with patients on haemodialysis to prevent thrombosis of the haemodialysis catheter. With patients suffering from heparin induced thrombocitopenia, heparinization is contraindicated and therefore, citrate is used as an alternative.

Materials and methods: A 72-year old female patient suffering from kidney failure attending haemodialysis for 25 years has been chosen for this report. Last year the patient was diagnosed with heparin induced thrombocitopenia and therefore catheter-locking in the interdialytic period was performed with citrate as the anticoagulant. Before the haemodialysis and immediately after the removal of the catheter-locking solution, blood sampling for routine laboratory tests was performed. Concentration of ionized calcium was determined potentiometrically using the point-of-care analyzer RapidLab 1265 (Siemens, Germany).

Results: The concentration of ionized calcium was measured prior to two haemodialysis treatments and the results were 0.33 mmol/L and 0.37 mmol/L, respectively. The patient, however, did not suffer from any symptoms of hypocalcemia. After the haemodialysis, ionized calcium was determined again, and its values were within the reference range (1.18–1.32 mmol/L).

Conclusion: The patient not suffering from any clinical symptoms of hypocalcemia and the concentration of ionized calcium being within the reference range after haemodialysis, indicates that a preanalytical error has occured. It is highly probable that the removal of the citrate solution from the catheter was not complete, which led to falsely decreased values of ionized calcium due to the capacity of citrate to chelate calcium ions.

P20-03

High-sensitive cardiac troponin T predicts survival in patients on chronic hemodialysis

Rajdl D (1), Racek J (1), Eiselt J (2), Trefil L (1), Malánová L (3)

(1) Charles University, Medical Faculty in Pilsen and University Hospital in Pilsen, Institute of Clinical Biochemistry and Hematology, Pilsen, Czech Republic
(2) Charles University, Medical Faculty in Pilsen and University Hospital in Pilsen, 1st Department of Internal Medicine, Pilsen, Czech Republic
(3) B. Braun Avitum, Dialysis center, Pilsen, Pilsen, Czech Republic

Corresponding author: rajdl@fnplzen.cz

Background and aims: Cardiac troponins and natriuretic peptide type B (BNP) use is well established in acute myocardial infarction and heart failure diagnosis. Recently, these markers (and especially cardiac troponins measured by high-sensitive assays) are emerging as new prognostic markers in wide variety of diseases. The goal of this study was to compare the prognostic utility of cardiac Trpoponin I (cTnI, Access 2, Beckman-Coulter), BNP (AxSYM 2, Abbott) and high-sensitive cardiac troponin T (hsTnT, Cobas e411, Roche) in hemodialysed (HD) patients.

Materials and methods: We analyzed cTnI, BNP and hsTnT levels in blood samples of 83 chronically HD patients (31 females; median age [interquartile range] = 65 [56-71] years). There were 37 deaths in our study population (median follow-time was 38.3 [17.0-54.0] months). We used Cox proportional hazard model to reveal the possible prognostic role of measured markers.

Results: Our results indicate that the best prediction of overall survival can be obtained from hsTnT (relative risk (RR) of overall mortality with its 95 % confidence intervals (CI): 1.022 [1.03 to 1.042], P = 0.03, followed by BNP (RR [CI]: 1.0001 [to 1.00004 to 1.001], P = 0.05. cTnI was not significantly associated with survival of HD patients in this setting.

Conclusion: High-sensitive troponin T and BNP can help in risk stratification of hemodialysed patients.

P20-04

Interpretation of cardiac markers in end stage renal disease patients in hemodialysis (HD)

Aakre K (1), Røraas T (2), Petersen P (2), Svarstad E (3), Sandberg S (1)

(1) Haukeland University Hospital, Laboratory of Clinical Biochemistry, Bergen, Norway
(2) University of Bergen, The Norwegian Quality Improvement of Laboratory Services in Primary Care (NOKLUS), Department of Public Health and Primary Health Care , Bergen, Norway
(3) University of Bergen, Renal Research Group, Institute of Medicine, Bergen, Norway

Corresponding author: kristin.moberg.aakre@helse-bergen.no

Background: The aim was to calculate the within-subject variation (CVi) and reference change value (RCV) for TnT and pro-BNP in HD patients.

Materials and methods: 19 patients (treated with HD for median 16.5 month) and 20 healthy voluntaries were included. Samples were collected once a week (immediately before dialysis and during morning hours, respectively) for ten weeks. Results below the detection limit of the high sensitive TnT and NT-proBNP assay (Modular Roche Diagnostics; 3 ng/L and 1 pmol/L) were excluded. CVi and RCVs were calculated after ln-transformation of the data.

Results: Half of the healthy controls were female and median age was 61 years. Median age of patients was 71 years, four patients were female. Eight patients had nephrosclerosis and six glomerulonephritis as main cause for renal disease. Four patients had clinical events during the ten week observation period and were excluded from calculations. Mean TnT and pro-BNP concentrations ranged from 18 to 189 ng/L and 22 to 6189 pmol/L, respectively in the HD patients. Most control subjects had TnT values below 3 ng/L (CVi could not be calculated) whilst mean NT-proBNP concentrations ranged from 2-63 pmol/L. In HD patients TnT CVi was 8.2%; TnT RCVs were -18% and +22%; NT-proBNP CVi was 26% and RCVs were -51% and +105%. In healthy controls NT-proBNP CVi was 55% and RCVs were -77% and +336%.

Conclusion: In cardiac stable HD patients TnT show less variation compared to NT-proBNP. The NT-proBNP variation in HD patients is lower as compared to healthy controls.

P20-05

The role of NGAL as predictor of delayed graft function after kidney transplantation

Glisic B (1), Lalic N (1), Naumovic R (2), Durutovic O (3), Majkic-Singh N (1)

(1) Clinical Center of Serbia, Center of Medical Biochemistry, Belgrade, Serbia
(2) Clinical Center of Serbia, Clinic for Nephrology, Belgrade, Serbia
(3) Clinical Center of Serbia, Clinic for Urology, Belgrade, Serbia

Corresponding author: biljaglisic@yahoo.com

Background: Delayed graft function (DGF) is very common complication after kidney transplantation which can increase the risk of early or late graft loss. Commonly, it is monitored by measuring the serum creatinin levels, unreliable parameter for assessing acute changes in graft function. Recently, NGAL has emerged as a novel biomarker for predicting the onset of DGF. We performed a pilot study to assess the role of NGAL, measured 3 hours after the transplantation, as a potential predictor of DGF.

Materials and methods: Our study included 20 kidney transplanted patients, divided into two groups: IGF (immediate graft function, 6 patients) and DGF (14 patients). NGAL was measured using the ARCHITECT® Urine NGAL assay (Abbott Diagnostics, Illinois, USA) in urine samples collected exactly 3 hours after the transplantation.

Results: The mean uNGAL values in IGF and DGF groups were 1215.3 ± 766.7 ng/mL and 1630.0 ± 1023.9 ng/mL, respectively. We found no statistically significant differences between the groups. After expressing the results as uNGAL to urinary creatinin ratio (NGAL/Cr), the differences were statistically significant ($P < 0.01$) with levels of 143.6 ± 79.4 ng/mg for IGF and 905.6 ± 656.7 ng/mg for DGF.

Conclusions: Our results have shown that NGAL measured in urine samples collected three hours after the transplantation can be used as a predictor of DGF, if expressed as NGAL-to-creatinine ratio.

P20-06

Nutritional status assessment in patients undergoing hemodialysis

Crnokrak S, Mujagić R, Honović L

General Hospital Pula, Clinical laboratory, Pula, Croatia

Corresponding author: slavica_crnokrak@net.hr

Introduction: Approximately 40 to 70 percent of patients with end-stage renal disease are malnourished. The assessment of nutritional status should be a routine care of dialysis patients to permit early recognition and the institution of appropriate therapy. Most of the standard methods of assessing nutritional status can be applied to patients with renal failure; however, some of these parameters are altered by uremia. There is no single measurement that can be used to determine the presence of malnutrition.

Materials and methods: Seventy male hemodialysis patients were included in this study. Nutritional parameters (body mass index, cholesterol, triglyceride, transfferin, albumin, pseudocholinesterase, uric acid, blood lymphocyte count) were measured by standard biochemical tests. Results were evaluated by a Forward stepwise multiple linear regression method.

Results: There were revealed an standard equation; body mass index (kg/m^2) = 1.795 × cholesterol (mmol/L) + 2.657 × transferrin (g/L); that shows a significant association of body mass index, especially, with cholesterol and transferrin (multiple $R = 0.675$; $P < 0.001$). However, body mass index were not correlated ($P > 0.05$) with zinc, albumin and pseudocholinesterase in serum and blood lym-

phocyte count. Thereafter, there were revealed an improved equation; body mass index (kg/m^2) = 0.016 × uric acid (μmol/L) + 0.756 × triglyceride (mmol/L) + 0.955 × cholesterol (mmol/L) + 1.875 × transferrin (g/L); that shows a higher correlation of body mass index (multiple R = 0.727; P < 0.001) with uric acid, triglyceride, cholesterol and transferrin.

Conclusion: We were suggested equation that may confidentially predict the nutritional status in patients undergoing hemodialysis.

P20-07

Visfatin is not associated with inflammatory markers in patients on hemodialysis

Ćelap I (1), Šimundić AM (1), Nikolac N (1), Miler M (1), Altabas K (2), Šefer S (2)

(1) University Hospital Center Sestre milosrdnice, Clinical Institute of Chemistry, Zagreb, Croatia
(2) University Hospital Center Sestre milosrdnice, Center for Hemodialysis, Department of Nephrology, Zagreb, Croatia

Corresponding author: ivana.celap@gmail.com

Background: Patients with chronic renal disease often suffer from diverse cardiovascular complications. Endothelial dysfunction has been involved in development of different forms of the cardiovascular diseases, including chronic renal disease. In recent years, ubiquitous adipokine called visfatin has been considered as a novel marker of endothelial dysfunction and inflammation. Thus, the aim of our study was to investigate the association of serum visfatin concentrations and well-established markers of cardiovascular risk in the patients on hemodialysis.

Materials and methods: Serum and plasma samples from 66 patients (40 males and 26 females) treated by hemodialysis were analysed for visfatin, fibrinogen, CRP and PAI-1 levels. Visfatin was determined by ELISA method while CRP, fibrinogen and PAI-1 were obtained by standard laboratory methods.

Results and conclusion: Visfatin did not correlate with the studied markers of inflammation (CRP (r = -0.05, P = 0.152); PAI-1 (r = -0.08, P = 0.558) and fibrinogen (r = 0.04, P = 0.772)). Statistically significant correlation between visfatin level and fibrinogen (r = 0.51; P = 0.008) and the time on dialysis (r = 0.70; P < 0.001) was observed in female patients. In conclusion, visfatin is not associated with CRP, fibrinogen and PAI-1 in patients on hemodialysis.

P20-08

Effect of hemodialysis on the expression of TLR-4 on monocytes

Dobrošević B (1), Bilandžija M (2), Barbić J (3), Šerić V (1)

(1) Clinical Hospital Centre Osijek, Department of Clinical Chemistry, Osijek, Croatia
(2) Clinical Hospital Centre Osijek, Department of hemodialysis, Osijek, Croatia
(3) University of Osijek, Medical School, Osijek, Croatia

Corresponding author: dobrosevic.blazenka@kbo.hr

Background: Infections are frequent complications in patients undergoing hemodialysis. End-stage renal disease is simultaneously associated with inflammation. It was recently shown that family of Toll-like receptors (TLR) play a critical role in innate immunity. It has been reported that expression of TLR-4 was lower in patients on dialysys. The purpose of this study was to determine the effect of hemodialysis on the expression of TLR-4 on CD14+ monocytes.

Materials and methods: In 33 hemodialysis patients, we measured expression of TLR-4 on CD14+ monocytes at the beginning (0 min.) and following (180 min.) hemodialysis. The hemodialysis procedure was performed by using polysulphone dialysers. Expression of TLR-4 on CD14+ monocytes was determined by staining with PE labeling anti-TLR-4 monoclonal antibody (eBioscience) and analyzed by flow cytometry (FACSCalibur, BD).

Results: The percentage of TLR-4 on CD14+ monocytes was significantly lower 180 min. after hemodialysis (21.7 ± 6.5) compared with beginning (24.8 ± 6.4 ; P < 0.01).

Conclusions: The expression of TLR-4 on monocytes becomes down-regulated in uremic patients. The hemodialysis procedure may suppress the expression of TLR-4.

P20-09

Markers of the obesity and inflammation in patients with metabolic syndrome and on dialysis

Miler M (1), Segulja D (2), Nikolac N (1), Celap I (1), Kackov S (3), Simundic AM (1)

(1) Sestre milosrdnice University Hospital Center, University Department of Chemistry, Zagreb, Croatia
(2) University Hospital Centre Zagreb, Department of Laboratory Diagnostics, Zagreb, Croatia
(3) Polyclinic Bonifarm, Medical biochemistry laboratory, Zagreb, Croatia

Corresponding author: marijana.miler@gmail.com

Background: Dialysis is an invasive treatment in chronic kidney failure associated with increased activation of inflammatory system. The aim of this study was to examine the difference between concentrations of classic inflammatory marker (C-reactive protein, CRP) and new markers from adipokine family (leptin and resistin) in patients on dialysis and with metabolic syndrome.

Materials and methods: Total of 140 patients were included in the study, 55 of them with metabolic syndrome (according to NCETP ATP III criteria), 66 on hemodialysis and 18 on peritoneal dialysis. For all patients body mass index (BMI) was provided. CRP concentration was determined with turbidimetric method on Beckman Coulter AU2700 analyzer (Beckman Coulter, Brea, USA). Concentrations of leptin and resistin were determined with fluorescent bead immunoassay (Bender MedSystems GmbH, Vienna, Austria) on the flow cytometer (Beckman Coulter, Brea, USA). Differences between 3 groups were tested with Kruskal-Wallis test.

Results: According to the results, CRP concentration was higher in group of dialyzed patients than in patients with metabolic syndrome ($P < 0.001$) whereas leptin and resistin concentrations did not differ between groups ($P = 0.115$ and $P = 0.569$, respectively). BMI was lower in group of patients on hemodialysis regarding to patients on peritoneal dialysis and with metabolic syndrome ($P < 0.001$).

Conclusions: CRP is increased in dialyzed patients and that probably indicates inflammatory response in dialysis. Since leptin and resistin concentrations are not increased in dialyzed patient, we hypothesize that those markers share some other regulatory mechanisms.

P21 - Toxicology and TDM

P21-01

Analytical validation of valproic acid on the Abbott Architect c8000 clinical chemistry system

Čepić K, Dujmov I, Mladina B, Šupe Domić D, Stanišić L

Split University hospital, Department of Clinical Chemistry, Split, Croatia

Corresponding author: katarina.cepic@kbsplit.hr

Background: Valproic acid is a broad-spectrum anticonvulsant drug. High concentration has been associated with hepatic toxicity and acute toxic encephalopathy. The aim of this study was to evaluate analytical performance of Abbott Architect c8000 analyzer for therapeutic drug monitoring (TDM) of valproic acid.

Materials and methods: Analytical validation of valproic acid determination by particle enhanced turbidimetric inhibition immunoassay (PETINIA) on Abbott Architect c8000 system included: inaccuracy (bias), within-run imprecision, between-run imprecision and method comparison with analytical system Architect i1000, CMIA method, for 66 human samples.

Results: Inaccuracy (bias) result was -0.24% to 2.4%. The highest coefficient of variation (CV) for

within run imprecision was 3.01% and beetwen-run imprecision was 2.82%. Linearity was confirmed with calibration curve in 6 points in concentration range from 12.5 to 150 µg/mL. Passing-Ba-block regression analysis of valproic acid comparison on two analyzers showed statistically significant, but clinically insignificant deviation in slope of regression equation (b = 0.9410; 95%CI = 0.9042-0.9766). The Cusum linearity test proved that there was a linear relationship between two methods.

Conclusion: Analytical validation of valproic acid by PETINIA method on Abbott Architect c8000 system fulfilled all previously established criteria and could be implemented in a routine laboratory work.

P21-02

The possible anti-cholinesterase properties of biologically synthesized platinum nanoparticles

Arowolo A, Whiteley C

Rhodes University, P.O. Box 94, Grahamstown, South Africa., Biochemistry, Microbiology and Biotechnology, Grahamstown, South Africa

Corresponding author: afolakesennuga@yahoo.com

Biomineralization, using protein cavity (cage) as a limiting growth field for the synthesis of uniformed sized and shaped nanoparticles (nps), is fast becoming a common approach in biological synthesis of nps. This is due to the fact that this method of production is by far simple, environmentally friendly and cost effective with great potential application in drug-delivery. However, little is known on the possible effect of these synthesized nps on the natural biological function of this protein cages. Also, the toxic effect of these nps on various biomedical target is unknown. In this work, spherical platinum nanoparticles (Pt-nps) of relatively uniform sized (3-5 nm) were biologically synthesized within the cavity of horse spleen apoferritin (HSA). Nanoparticles were characterized using UV, Transmission electron microscopy (TEM) and Electron dispersion analysis of X-rays (EDAX). The effect of synthesized Pt-nps on the ferroxidase activity of HSA was investigated. Finally, their potential anti-cholinesterase or neurotoxic properties were also studied. Synthesized Pt-nps significantly increased the ferroxidase activity of HSA by up to 9-fold. Results showed further, no significant inhibition on AChE activity compared to about 80% inhibition earlier reported with chemically synthesized nanoparticles. Pt-nps are well known to be efficient catalyst and may explain the rapid increase in the ferroxidase activity of HSA observed. We also believe the protein shell of HSA shielded the Pt-nps from eliciting any significant effect on the activity of AChE.

P21-03

Recreational drugs in clinical toxicology: new challenges in the everyday routine

Lakatos A (1), Lajtai A (1), Porpaczy Z (2), Mayer M (2), Kovacs G (1)

(1) University of Pécs, Med. School, Dept. of Laboratory Medicine, Pécs, Hungary
(2) University of Pécs, Med. School, Dept. of Forensic Medicine, Pécs, Hungary

Corresponding author: agnes.lakatos@aok.pte.hu

In clinical laboratory toxicology we use biological samples (mostly urine) of the poisoned patient to confirm the consumed dangerous substances. The anamnesis in this field is usually inefficient. The patients are often unconscious or don't want to tell or don't even know what was the substance they used for suicide or abuse. The spectra of most often used poisonous agents are changing in the time. It is also true for the medical drugs but in the field of the recreational drugs weekly appearing and explosively spreading new substances result everyday challenge for the clinical toxicologists. Not to be illegal for a while helps diffusing the usage of them. The lack of experience of the application of emerging designer drugs often draw the user to run into serious status and need emergency medical treatment. Neither the widespread immunological rapid tests, nor the medical experi-

ence can help the diagnosis of them. We use HPLC-DAD (Shimadzu TOX.I.S) system for toxicological screening and identifying the poisonous basic drugs. Its library can be expanding by the user. To validate the „reference materials" we use MALDI TOF method. We have managed to analyze mephedrone, flephedrone, 4-MEC, MDPV, Benzo-fury (5APB), 4FA, pentedrone and methoxetamine in the last 2 years in increasing number (about 300) of clinical cases. The HPLC method is convenient for the detection and identification of a broad spectrum of drugs it can be either well known or new.

P21-04

Cannabimimetics – A new challenge in routine clinical toxicology

Grobosch T, Binscheck T

Labor Berlin - Charité Vivantes GmbH, Laboratoriumsmedizin & Toxikologie, Berlin, Germany

Corresponding author: thomas.grobosch@laborberlin.com

Background: A 24-yr-old woman with a history of anorexia nervosa/bulimarexia was found unresponsive, breathless and asystolic in bed. After 30 min reanimation attempts of the emergency doctor the patient was defibrillated and transported to hospital. On admission, the EGC showed a prolonged QT interval and developed a significant metabolic acidosis with severe hypokalaemia.

Materials and methods: Systematic toxicological analysis: CEDIA, HPLC-DAD, HS-GC. Qualitative Screening (XLC-QQTOF). Quantitative analysis: Cannabimimetics, fentanyl, midazolam (LC-MS/MS), amiodarone (LC-MS).

Results:

Urine: Drug screening by CEDIA was positive for benzodiazepines. Additional analytics, including diuretics, laxantia, etc. resulted all negative. XLC-QQTOF analysis identified midazolam, fentanyl, and the N-(5-hydroxypentyl) metabolite of JWH-018 (110 µg/L). The quantitative LC-MS/MS analysis for cannabimimetics identified another metabolite of JWH-018, N-pentanoic acid (43 µg/L).

Plasma: STA revealed midazolam (640 µg/L). XLC-QQTOF analysis identified midazolam, amiodarone and fentanyl. Additional quantitative analysis of the plasma sample identified JWH-018 (58 µg/L), the N-pentanoic acid metabolite (40 µg/L). Three days after reanimation, the neuron specific enolase in serum was significantly increased (80 µg/L) - indicating hypoxic brain damage.

Conclusions: Clinicians, and the users need to be aware of the severe clinical effects following consumption of synthetic cannabinoid preparations marketed as partly legal cannabis alternatives. The cannabinomimetics cannot be detected by commonly used immunoassays for THC, therefore mass spectrometry is essential for identification. It is not clear whether the disturbance of myocardial repolarisation in this case was specifically induced by JWH-018 or might be facilitated by other specific circumstances in this case.

P21-05

Extreme case of designer drug abuse

Lajtai A (1), Lakatos A (1), Benkő A (2), Mayer M (2), Porpáczy Z (2), Kovács L. G (2)

(1) University of Pécs, Medical School, Department of Laboratory Medicine, Pécs, Hungary
(2) University of Pécs, Medical School, Department of Forensic Medicine, Pécs, Hungary

Corresponding author: agnes.lakatos@aok.pte.hu

The identification of new designer drugs means the largest problem in the clinical and forensic toxicology examinations; reference materials are not available, their metabolisms are unknown, their versatility is unlimited. The medical condition of a drug user is frequently critical, requiring intensive care, not to mention the possibility of long-term adverse reactions. Since January 2012 nine designer drugs became illegal in Hungary (e.g. MDPV, 4- Fluroamphetamin, some of the

JWH compounds). Illicit marketing and abuse of these compounds seem to be reduced, in contrast to those drugs which are still legal but we haven't met them before in our diagnostics practice (e.g. tryptamine derivates, 3-FA, pentedrone). In the near past, we analyzed serum and urine samples of a 27 year old chronic male drug user (GC, Abbott Axsym, HPLC-DAD: Shimadzu TOX.I.S). In the urine sample we detected several kinds of drugs in large quantities. The main component was pentedrone. However, the interpretation of the results was not simple. The complication was caused by some unknown peaks that appeared on the chromatogram. These peaks are likely to be the products of the metabolic reduction of pentedrone. Spectra of these metabolites are very similar to those of ephedrine, but their retention times are different. In conclusion, consumers of various designer drugs might be viewed as the participants of a pharmaceutical study, who voluntarily accept to take part in a dangerous and uncontrolled experiment and help us to discover new drugs,metabolic routes and adverse effects.

P21-06

Identification of acidic compounds in acute intoxications by on-line SPE-HPLC-DAD

Mut L (1), Grobosch T (1), Binscheck T (1), Frenzel W (2)

(1) Labor Berlin - Charité Vivantes GmbH, Laboratory Medicine and Toxicology, Berlin, Germany
(2) Berlin Institute of Technology, Department of Environmental Technology, Berlin, Germany

Corresponding author: SwetlaMut@t-online.de

Background: Most therapeutic drugs relevant in clinical toxicology are characterized by basic and neutral properties, but a number of acidic medications (NSAID's, barbiturates) can cause life-threatening poisonings. Therefore, an on-line SPE-HPLC-DAD screening method was developed to identify acidic compounds in poisonings. An in-house library was generated to support this method.

Materials and methods: To reduce matrix effects and to deproteinate the plasma, precipitants (acetone, methanol), ratios (1/2, 1/3, 1/4, 2/3, 3/4 plasma/precipitant), buffers (pH: 6, 2.3, 9), injection volumes (0.25 mL, 0.50 mL, 1.0 mL) were tested for maximum performance. Four SPE cartridges were examined in terms of extraction efficiency of acidic compounds. Six HPLC-columns were studied for optimum peak intensity and symmetry. Gradients for an on-line extraction and chromatographic separation of acidic compounds were created. Data of analyzed substances and poisonings were used to establish an in-house library. The method was applied to acute intoxications.

Results: Optimal results for sample preparation were precipitation of plasma with acetone (1/2), dilution of supernatant in buffer (pH = 6) and injection volume: 1.0 mL. Only the SPE-column StrataX-A (pH = 6), allowed the extraction of acids. The most appropriate column for analytical separation and peak symmetry was a 150 x 4.6 mm, 3 µm C6-Phenyl column. The analysis time was 44 min., including on-line extraction (12 min). An in-house library included > 150 entries of acidic compounds and metabolites. Such substances as salicylic acid, or ibuprofen were identified in intoxications.

Conclusion: The described method proved to be efficient and sensitive for screening of acidic therapeutic drugs in human plasma in case of acute poisonings.

P21-07

Automated online solid phase extraction UPLC/MS/MS for the analysis of mycophenolic acid

Eastwood M (1), Chusney G (2), Calton L (1)

(1) Waters Corporation, Clinical, Manchester, United Kingdom
(2) Hammersmith Hospital, Imperial College Renal & Transplant Centre, London, United Kingdom

Corresponding author: martin_eastwood@waters.com

Background: The recent consensus report evaluating therapeutic drug monitoring of mycophenolic acid (MPA) highlighted the need for accurate drug dosing strategies to minimize the incidence of drug-related toxicity while maintaining efficacy. Here we evaluate the potential of a new online solid phase extraction (SPE) system coupled to UltraPerformance liquid chromatography tandem mass spectrometry (UPLC/MS/MS) for the automated sample preparation and analysis of MPA in human plasma.

Materials and methods: Commercially available kits were used for calibrators and QC material. Method comparison was carried out using anonymized patient samples quantified using a validated LC/MS/MS assay. All samples were pre-treated with zinc sulphate and methanol. Automated online extraction was carried out with a Waters® ACQUITY UPLC coupled to a Waters MassTrak Online SPE Analyzer* and analysed using a Waters ACQUITY® TQD mass spectrometer.

Results: Following CLSI-EP6-A, the assay was shown to be linear from 0.01–50 µg/mL (N = 5). Coefficients of variation for inter- and intra-assay imprecision for low (1.94 µg/mL), mid (2.35 µg/mL), high (5.5 µg/mL) QC samples were all < 10% (N = 25, days = 5). Method comparison using patient samples previously analyzed with a validated LC/MS/MS assay (N = 50) was described by the Deming equation y = 0.99x-0.01. Compared with conventional one-dimensional chromatography, matrix effects were reduced by the online SPE as determined qualitatively by targeted multiple reaction monitoring of phospholipids and post-column infusion of analytes.

Conclusions: We have successfully quantified mycophenolic acid utilising automated online SPE with UPLC/MS/MS. The assay demonstrates good linearity, precision and accuracy with minimal ion suppression.

*NOTE: The MassTrak Online SPE Analyzer is under development

P21-08

Automated online solid phase extraction UPLC/MS/MS for simultaneous analysis of immunosuppressants

Eastwood M, Balloch S, Cooper D, Calton L

Waters Corporation, Clinical, Manchester, United Kingdom

Corresponding author: martin_eastwood@waters.com

Background: Therapeutic drug monitoring of immunosuppressants is an important requirement for the management of transplant patients. To streamline workflow there is a demand for the simultaneous measurement of multiple analytes. Here we evaluate the potential of online solid phase extraction (SPE) coupled to UltraPerformance liquid chromatography tandem mass spectrometry (UPLC/MS/MS) for the automated sample preparation and simultaneous analysis of cyclosporin A (CsA), tacrolimus, sirolimus and everolimus.

Materials and methods: Commercially available kits were used for calibrators and QC material. Samples obtained from the ASI Ltd International Proficiency Testing Scheme (IPT) were used to assess accuracy. All samples were pre-treated with zinc sulphate and acetonitrile. Automated online extraction was carried out with a Waters® ACQUITY UPLC coupled to a Waters MassTrak Online SPE Analyzer* and analysed using a Waters ACQUITY® TQD mass spectrometer.

Results: The assay was linear from 24.8–1515 ng/mL for CsA, 1.0–31.6 ng/mL for tacrolimus, 0.9–30.1 ng/mL for everolimus and 0.9–27.7 ng/mL for sirolimus, with r2 values > 0.997 (N = 5). Inter- and intra-assay imprecision for low, mid, high QCs were all < 10% CV (N = 5 *per* analyte). IPT samples were all within 10% of expected values (N = 5 per analyte). Compared with conventional one-dimensional chromatography, matrix effects were reduced as determined qualitatively by targeted multiple reaction monitoring of phospholipids and post-column infusion of analytes.

Conclusions: We have successfully quantified CsA, tacrolimus, sirolimus and everolimus simultaneously utilising automated online SPE with UPLC/MS/MS. The assay demonstrates good linearity, precision and accuracy with minimal ion suppression.

*NOTE: The MassTrak Online SPE Analyzer is under development

P21-09

Development of a LC-MS/MS method for the measurement of propofol and propofol glucuronide

Pitkin S, Zaman M, Benton S

Barts Health NHS Trust, Department of Clinical Biochemistry, London, United Kingdom

Corresponding author: sarah.pitkin@bartshealth.nhs.uk

Background: Propofol is an intravenous hypnotic agent used for sedation in intensive care units. A potentially fatal adverse effect is 'propofol-related infusion syndrome' (PRIS). There is interest in whether risk factors for PRIS relate to changes in metabolism of propofol. The aim was to develop and validate an LC-MS/MS method for the measurement of propofol and propofol glucuronide in whole blood, suitable for pharmacokinetic studies.

Materials and methods: Freeze-thawed whole blood was spiked with internal standards (propofol-d17; propofol glucuronide-d17) and proteins precipitated using acetone. Supernatant was heated for 10mins at 60 °C with dansyl chloride (2.7 mg/mL) and ammonium hydroxide (0.03N). The reaction mixture was injected on to a Waters Acquity UPLC and Quattro Premier XE tandem mass spectrometer. Gradient elution was followed by quantification by electrospray ionisation mass spectrometry in multiple reaction monitoring mode.

Results: Dansyl chloride derivatisation significantly increased the detection of propofol. Propofol glucuronide did not react with dansyl chloride and was quantified in its underivatised state. The run time was 11.5 mins. Standard curves were linear (r^2 > 0.99) across the calibration ranges. The intra- and inter-assay coefficients of variation were < 15% (N = 9-10) and the lower limits of quantitation for propofol and propofol glucuronide were 0.1 µg/mL and 0.25 µg/mL respectively. No ion suppression or enhancement or carry-over was observed. The parent drug and its metabolite were detectable in whole blood from patients receiving propofol infusions.

Conclusions: A novel LC-MS/MS method for the simultaneous measurement of propofol and propofol glucuronide was developed, which will be of use in the further investigation of PRIS.

P21-10

Oxidative stress/serum acetylcholinesterase in Nigeria organophosphate pesticide exposed farmers

Akinosun O (1), Adewole T (2)

(1) College of Medicine, University of Ibadan, Chemical Pathology Department, Ibadan, Nigeria
(2) University College Hospital, Chemical Pathology Department, Ibadan, Nigeria

Corresponding author: bayoakin2002@yahoo.com

Background: Organophosphate agents constitute about half of all pesticides used globally and they appear to pose the greatest risk among all the pes-

ticides. Despite significant advances in the understanding of the potential mechanism of toxicity in intentional exposures, the precise health effects following occupational exposures are yet to be completely defined.

Materials and methods: Oxidative stress status and Acetylcholinesterase activities were studied in blood samples obtained from 25 farmers in Idi Ayunre, Oluyole local government area of Oyo State, using organophosphate (OP) pesticides in spraying their cash crops (cocoa and cola nut trees) with a minimum work history of 10 years, in the age range of 35-75 years. 20 age-matched workers, who never had any exposure to OP pesticides were selected as controls in Ibadan, the capital of Oyo State. Total Plasma Peroxide (TPP) levels using FOX-2 reagent, Total Anti oxidant potential (TAP) using the ferric reducing antioxidant power (FRAP) assay were determined and oxidative stress index (OSI) an indicator of oxidative stress status was calculated. Blood acetylcholinesterase activity was measured using HPLC.

Results: Statistically significant decrease in the mean blood levels of acetylcholinesterase (IU/L) in the farmers (43.35 ± 9.07) compared to the controls(65.28 ± 7.66). TPP (umol H_2O_2/L) increased significantly in the farmers (14.32 ± 5.18) than in the controls (10.25 ± 3.60) ($P < 0.05$), while depletion of TAP (umolTroloxequiv/L) was observed in the farmers (915.65 ± 130.16) than the controls (975.80 ± 142.70). OSI(%) in farmers (1.65 ± 0.69) increased significantly than controls (1.08 ± 0.39).

Conclusion: OP pesticides users are exposed to increased oxidative stress. Assay of acetylcholinesterase activities could be a good biomonitoring index.

P21-11

Blood mercury concentrations in 3 cities in Spain

Trasobares E (1), González -Estecha M (1), Martínez García M (2), Herbello Hermelo P (3), Bermejo Barrera P (3), Guillén Pérez J (4)

(1) Hospital Clínico San Carlos, Laboratory Medicine, Madrid, Spain
(2) Universidad Politécnica de Cartagena, Ingeniería Química y Ambiental, Cartagena, Spain
(3) Universidad de Santiago de Compostela, Química Analítica, Santiago de Compostela, Spain
(4) Dirección General de Salud Pública, Región de Murcia, Dirección General de Salud Pública, Región de Murcia, Cartagena, Spain

Corresponding author: montse@cmpx.net

Background: There is increasing concern about the effects of exposure to methylmercury in adults. The aim of this multicenter study is to measure blood mercury concentrations in an adult population in 3 cities in Spain.

Materials and methods: We recruited 792 employee volunteers from 2 hospitals and one University in Madrid, Cartagena and Santiago de Compostela. Blood mercury concentration (µg/L) was measured in Madrid by cold vapour atomic absorption spectrometry in a Perkin Elmer FIMS 400 and in Cartagena in a direct manner in a DMA-80 Millestone based on the EPA 7473 method. Evaluation of concordance by Bland Altman plot was performed between these two methods. Blood mercury in Santiago de Compostela was determined by Cold Vapour Atomic Absorption Spectrometry in a Perkin Elmer 4100 equipped with a FIA Perkin Elmer 400.

Results: Upon evaluation of concordance between Madrid and Cartagena methods, almost all the measurements concorded and were included in the 95% confidence interval of the mean of the differences. The medians of blood mercury (µg/L) obtained were: Madrid (7.9; IQR: 5.2-11.5); Cartagena (8.95; IQR: 6.7-13.8) and Santiago de Compostela (15.1; IQR: 10.2-19.9). A statistically significant difference was observed among blood mercury concentrations in the 3 cities ($P < 0.001$). We also observed statistically significant differences between Madrid

and Cartagena (P = 0.004); Madrid and Santiago de Compostela (P < 0.001) and between Cartagena and Santiago de Compostela (P < 0.001).

Conclusions: Higher blood mercury concentrations were found in Spain than those previously reported in other European countries, probably due to the higher fish consumption in Spain.

P21-12

Influence of immunosuppressive regimen change on renal function in liver transplant recipients

Ožvald I (1), Mrzljak A (2), Radisic Biljak V (1), Perkov S (1), Flegar Mestric Z (1)

(1) University Hospital Merkur, Institute of Clinical Chemistry and Laboratory Medicine, Zagreb, Croatia
(2) University Hospital Merkur, Department of Gastroenterology, Zagreb, Croatia

Corresponding author: iozvald@gmail.com

Background: Liver transplantation is accepted treatment of choice for many liver diseases. Long–term survival is limited by toxicity of immunosuppressive agents, sub-clinical as well as cronic rejection. Newer classes of immunosuppressive agents, including calcineurin inhibitors CNI (cyclosporine and tacrolimus) and mammalian target of rapamycin (mTOR) inhibitors (sirolimus and everolimus) have potential to improve long-term outcomes. Many long-term survivors face a considerable risk of renal dysfunction due to CNI. The aim of the study was to determine benefits of sirolimus compared to CNI towards kidneys toxicity.

Materials and methods: We have monitored nine orthotopic liver transplantation patients (OLT) who underwent conversion of immunosuppressive regiment from CNIs to sirolimus. Creatinine concentrations were measured with the creatinine enzymatic assay. Measured concentrations of serum creatinine were used to estimate renal function, after dosing CNIs and converting to sirolimus, each one, three and six months after the dose.

Results: Referring to the long-term outcomes of OLT patients following results could be seen: Among all patients switched to sirolimus, the levels of serum creatinine (80.3 µmol/L ± 12.8 *vs*. 76.0 µmol/L ±12.7) remained stable in three of them, whereas the levels of serum creatinine started to decrease (119.4 µmol/L ± 29.8 *vs*. 83.2 µmol/L ± 26.3) after administration of sirolimus in six of the patients.

Conclusion: These preliminary results have shown that sirolimus is effective in preventing rejection in OLT recipients and it is associated with improved renal function. mTOR inhibitors might have a role as an early alternative to CNIs in patients with CNI nephrotoxicity.

P21-13

Multi-drug intoxication fatality involving atorvastatin: a case report

Cibicek N (1), Caran T (2), Pribyl P (3), Dobias M (4), Ondra P (4), Vorisek V (5)

(1) Palacky University Olomouc, Faculty of Medicine and Dentistry, Department of Medical Chemistry and Biochemistry, Olomouc, Czech Republic
(2) Hospital Hranice, Department of Anaesthesiology and Resuscitation, Hranice, Czech Republic
(3) Hospital Hranice, Department of Internal Medicine, Hranice, Czech Republic
(4) University Hospital Olomouc, Institute of Forensic Medicine and Medical Law, Olomouc, Czech Republic
(5) Charles University in Prague, Faculty of Medicine and University Hospital in Hradec Kralove, Institute of Clinical Biochemistry and Diagnostics, Hradec Kralove, Czech Republic

Corresponding author: norbert.cibicek@upol.cz

Background: Mixed antihypertensive drug intoxication poses a significant risk for patient mortality. In tandem to antihypertensives, hypolipidemic medicines (especially statins) are often prescribed. Among their adverse effects belongs rhabdomyolysis.

Case description: We report a case of fatal multidrug overdose in a 65-year-old female alcoholic. The woman was admitted to a municipal hospital unconscious. Empty blister packs indicated the abuse of 250 tablets of urapidil, 42 tablets of vera-

pamil/trandolapril, 50 tablets of moxonidin, 80 tablets of atorvastatin and 80 tablets of diacerein. Standard measures (gastric lavage, mechanical ventilation, massive doses of vasopressors, volume expansion, diuretics and alkalinisation) failed to provide adequate drug elimination and hemodynamic support. The patient deceased on the fourth day.

Results: Dramatic elevations of serum myoglobin (34880 ug/l) and creatine kinase (281 ukat/l) were accompanied by rise in cardiac troponin I and creatinine. Gas chromatography revealed ethanol 1.17 g/kg (blood) and 2.81 g/kg (urine). Thin layer chromatography and gas chromatography of gastric content and urine verified verapamil, moxonidin and urapidil fragment (diacerein method was unavailable). Atorvastatin and trandolapril concentrations (LC-MSn) equaled 277.7 ug/L and 57.5 ug/L, resp. (serum) and 8.15 ug/L and 602.3 ug/L, resp. (urine). Histology confirmed precipitates of myoglobin with acute necrosis of proximal renal tubules in association with rhabdomyolysis of striated muscle and myocardial dystrophy.

Conclusions: Distributive and cardiogenic shock in conjunction with acute renal failure due to the combined self-poisoning with vasoactive agents and atorvastatin were determined to be this decedent's immediate cause of death. The manner of death was assigned to be suicidal.

P21-14

Pharmacokinetics of mycophenolic acid in renal allograft recipients: role of ABCC2 polymorphisms

Lalić Z (1), Božina N (1), Nađ Škegro S (2), Trkulja V (3), Lovrić M (1), Granić P (1)

(1) University Hospital Center Zagreb, Department of Laboratory Diagnostics, Zagreb, Croatia
(2) University Hospital Center Zagreb, Department of Urology, Zagreb, Croatia
(3) Zagreb University School of Medicine, Department of Pharmacology, Zagreb, Croatia

Corresponding author: lovricm@kbc-zagreb.hr

Background: Mycophenolic acid (MPA) displays large between- and within-subject pharmacokinetic variability. MPA is metabolized by UGTs to inactive 7-O-MPA-glucuronide (MPAG). MPA and MPAG are subject to enterohepatic recirculation. Biliary and kidney excretion of MPA/MPAG involves several transporters, including multidrug resistant protein-2 (MRP-2) coded by polymorphic *ABCC2*, which can influence MPA pharmacokinetics. The objective of this study was to perform MPA pharmacokinetics et steady state conditions during one dosing interval (12 h), in 68 renal allograft recipients. Pharmacokinetic variability in relation to donor and recipient *ABCC2* genotypes is estimated.

Patients and methods: Blood samples were drawn at 0, 0.5, 1, 2, 3, 8, and 12 h after the morning dose. Genotyping of *ABCC2* C-24T and G1249A was performed using TaqMan-based allele-specific PCR assay. Plasma concentrations of MPA were determined using validated HPLC method.

Results: Pharmacokinetic parameters: Cmax,ss (mg/L) 12.3 ± 6.7 ; Tmax (hrs) 2 (0.2-12); AUCt,ss (mg*h/L) 39.9 ± 20.7; Cmin,ss (mg/L) 1.3 ± 1.2; Trough 1 (time 0) (mg/L) 2.7 ± 2.1; Trough 2 (time 12) (mg/L) 1.9 ± 1.9. Considering *ABCC2* genotypes associations were found between: donor C-24T and lower through1 concentrations in T allele carriers ($P = 0.003$); G1249A variants and lower Cmax/dose in A allele carriers ($P < 0.05$), -24T allele carriers and % of concentration swing (OR 1.88, 95%CI 1.09-3.23). For recipient genotypes correlations were between: 1249A allele and lower Cmin and trough2 concentrations, and higher % of concentration swing (OR 1.85 95%CI 1.05-3.28).

Conclusion: The pharmacokinetics of MPA is affected by the *ABCC2* polymorphisms.

P21-15

HPLC of carbohydrate-deficient transferin and more sialylated transferrin glycoforms in children

Bianchi V (1), Raspagni A (2), Arfini C (3), Vidali M (4)

(1) SSS Antonio e Biagio Hospital, Toxicology Laboratory, Alessandria, Italy
(2) Insubria University, School of Medicine, Varese, Italy
(3) SS Antonio e Biagio, Clinical Laboratory, Alessandria, Italy
(4) Maggiore della Carità Hospital, Clincal Chemistry Unit, Novara, Italy

Corresponding author: vbianchi@ospedale.al.it

Background: The evaluation of age-specific distribution of transferrin glycoforms in paediatric patients may help in defining reference intervals which are critical for an improved and earlier diagnosis.

Materials and methods: Serum samples from 224 children (age: 2 months-14 years) were analyzed by HPLC (CDT by HPLC kit, Bio-Rad, Munich, Germany) and glycoforms expressed as percentage of the total area of transferrin (Tf).

Results: Asialo- and Monosialo Tf were not detectable in any patient. Median (IQR) were respectively 0.92% (O.80-1.04%) for DisialoTf; 3.47% (2.69-4.18%) for Trisialo-Tf; 82.54% (81.32-83.53%) for Tetrasialo-Tf; 12,73% (11.91-14.09%) for Pentasialo-Tf. Statistically significant differences in Trisialo-Tf ($P < 0.001$), Tetrasialo-Tf ($P = 0.001$), Pentasialo-Tf ($P < 0.001$), but not in Disialo-Tf, were observed between the age groups.

Conclusions: Age-specific Diaisialo-Tf cut-offs are not necessary. In children 1.3% and 6.4% may be suggested as upper limits of normal range to detect increases of Disialo- and Trisialo-Tf. The presence of Asialo- and Monosialo-Tf should be considered an abnormal finding and prompt further investigations.

P22 - Vitamin D - PTH

P22-01

Comparison of total 25-OH vitamin D automated immunoassays versus LC-MS/MS

Bausela C, Gaviña B, Ortega I, Torrejón M, Arroyo M

Hospital Clínico San Carlos, Análisis Clínicos, Madrid, Spain

Corresponding author: cris_bausela@hotmail.com

Background: Vitamin D has an important role in both calcium homeostasis and bone metabolism .The best serum marker to determine nutritional status is Total 25-OH Vitamin D. The great methodological variability and the lack of international 25-OH Vitamin D standards for immunoassays, take to wrong classifications, treatments and monitoring. The aim of this study is to compare three automated immunoassay methods for the measurement of Total 25-OH vitamin D (Abbot, Roche and DiaSorin) with LC-MS/MS.

Material and methods: Human serum samples (N = 150) were measured with the followings methodologies:

- DiaSorin LIASION 25-OH VITAMIN D TOTAL CLIA®
- Abbot Diagnostic ARCHITECT 25-OH VITAMIN D CMIA®
- Roche ELECSYS 25-OH VITAMIN D TOTAL ECLIA®
- LC-MS/MS ASSAY

Results obtained by the three immunoassays were compared with LC-MS/MS by linear correlation plots and concordance correlation coefficients. Weight Kappa Coefficient was used to determine the agreement between different assays. All data were analyzed with SPSS software (version 15.0).

Results: Spearman´s Rho Coefficients: DiaSorin = 0.840, Abbot = 0.848 and Roche = 0.817. Intraclass Correlation Coefficients, ICC: DiaSorin = 0.931, Abbot = 0.913 and Roche = 0.897. Weight Kappa Coefficients: DiaSorin = 0.701, Abbot = 0.734, Roche = 0.718.

Conclusions: Correlation and concordance coefficients between the three immunoassays and LC-

MS/MS is good and shows that immunoassays methods are equivalent. However, individual comparison of some data shows that patients can be classified in a different group, according with the method used in the measurement. Therefore, each laboratory should establish their own cutoff points.

P22-02

Stability of PTH in blood specimens after delayed processing

Fernandez Leivas A, Garcia Moreira V, Laborda Gonzalez B, Martinez Gago M, Garcia Alonso S, Fernandez Rodriguez E

Hospital de Cabueñes, Servicio de Analisis Clinicos, Gijon, Spain

Corresponding author: anafleivas@hotmail.com

Background: The parathyroid hormone is secreted by the parathyroid gland (homeostasis of calcium and phosphorus). The recommended preanalytical processing for iPTH is cold centrifugation the sample immediately after collection and frozen until analysis. The aim of our study was to analyze the variability in the results of iPTH comparing two preanalytical protocols.

Materials and methods: 62 serum samples were collected in duplicate and iPTH was measured by electrochemiluminescence immunoassay: autoanalyzer Cobas e411 (Roche). The samples were processed with different preanalitical conditions: immediate centrifugation and freezing (group 1, reference) and sample at room temperature ≥ 2h, centrifugation and frozen until analysis (group 2, study). Significant differences were determined by Student paired-t, using the software MedCalc. Significance was set at $P < 0.05$.

Results: The mean iPTH group1 was 85.07 pg/mL (95%CI: 66.35-103.79) while iPTH in the group2 was 84.54 pg/mL (95%CI: 65.42-103.65). There was no significant difference between groups (paired t-test, $P > 0.05$). The coefficient of variation described by the commercial insert of the method (also verified by our laboratory) is 4.17 and the obtained according to the duplicate samples of the study between two groups was 4.30, so no differences were observed.

Conclusions: According to results, in the iPTH cuantification is unnecessary the immediate centrifugation of the sample. The determination may be made in the same serum tube used to analyze the different biochemical parameters, whose centrifugation is usually done between 2 and 3 hours after collection; this protocol can avoid preanalytical errors, saving time and expenditure rationalization.

P22-03

Evaluation of automated method for measurement of serum 25-hydroxyvitamin D

Vogrinc Ž, Lovrić M, Sertić J

Clinical Hospital Center Zagreb, Clinical Institute of Laboratory Diagnostics, Zagreb, Croatia

Corresponding author: zvogrinc@kbc-zagreb.hr

Background: The most reliable indicator of vitamin D status in organism is measurement of circulating 25-hydroxyvitamin D (25(OH)D) in serum or plasma. The measurement is challenging because 25(OH)D is highly lipophylic, bound strongly to protein, present in low concentrations and exsists in two structurally similar forms, $25(OH)D_3$ and $25(OH)D_2$. The aim of the study was to evaluate a new automated assay for quantitative determination of serum total 25(OH)D (Roche Diagnostics) and compare it with selective and sensitive HPLC method with UV detector.

Materials and methods: 25(OH)D from human sera was measured using two methods: fully automated competitive electrochemiluminiscence method (Roche Elecsys Vitamin D total assay) and Chromsystems HPLC method for 25(OH) D_3/D_2. The evaluation protocol consisted of within-run imprecision (10 sequential runs) and between-run imprecision (10 consecutive working days, 2 sequential runs) with commercial controls PreciControl

Bone 1 and 2, inaccuracy (N = 20), and method comparison (routine serum samples, N = 37). Methods were compared by Passing and Bablok regression and Bland-Altman analyses.

Results: Within-run imprecision for Roche Elecsys Vitamin D total assay was 2.56%, and between-run imprecision was 2.99% and 3.79%. Quality requirement for inaccuracy was fulfilled. The comparison with HPLC method demonstrated strong correlation (r = 0.9581; y=0.918x-4.5082) and good agreement (bias = ± 1.96 SD).

Conclusion: Roche Elecsys Vitamin D total assay showed good correlation and agreement with HPLC-UV method and represents an accurate and precise automated tool for serum total 25(OH)D determination.

P22-04

Vitamin D status in heart failure patients is dependent on the assay method used

Silva N (1), Martins S (2), Tuna D (2), Lourenço P (3), Bettencourt P (4), Guimaraes J (1)

(1) Faculty of Medicine, University of Porto & Sao Joao Hospital, Biochemistry Department & Clinical Pathology Department, Porto, Portugal
(2) Sao Joao Hospital, Clinical Pathology Department, Porto, Portugal
(3) Sao Joao Hospital, Internal Medicine Department, Porto, Portugal
(4) Faculty of Medicine, University of Porto & Sao Joao Hospital, Internal Medicine Department, Porto, Portugal

Corresponding author: jtguimar@med.up.pt

Background: Heart failure (HF) is a prevalent public health problem. Studies indicate a beneficial role for Vitamin D (VTD) on cardiovascular health. Knowing that VTD measurement is highly method dependent, our aim was to evaluate how two different automated assays influence VTD status classification in HF patients.

Materials and methods: Serum samples from 134 patients (65 males and 69 females with 75 ± 13 years old) were evaluated using two chemiluminescent immunoassays (Roche Cobas®- routine method, and Abbott Architect®). Three groups were set: ≤ 10 ng/mL for deficient (A); 11-20 ng/mL for insufficient (B) and > 20 ng/mL for optimal (C). The statistical analysis was performed in Medcalc® software.

Results: For Architect® the values ranged from 5.2 ng/mL to 29.8 ng/mL with a mean value of 14.3 ng/mL. For Cobas® ranged from 3.0 ng/mL to 23.1 ng/mL with a mean value of 8.2 ng/mL. The correlation coefficient was 0.8295, with a mean difference of 6.0 ng/mL, (95%CIs, [5.5; 6.7], P < 0.001). When evaluated on Architect®, 73 (54%) of the patients changed VTD group status: 59 from A to B and 14 from B to C. From the 32 samples which had a value of < 3.0 ng/mL (LOD) on Cobas®, 21 patients continued in A group while 11 changed to B. The mean difference for each group was 6.1, 6.1 and 6.4 ng/mL, respectively.

Conclusion: Pathologists and clinicians must be aware of the clinical consequences that method selection has on patients Vitamin D status classification.

P22-05

Comparison of vitamin D3 and total vitamin D values

Županić D, Crnokrak S, Juričić G, Jedrejčić K, Honović L

Pula General Hospital, Department of Laboratory Diagnostics, Pula, Croatia

Corresponding author: danijela.zupanic@pu.t-com.hr

Introduction: Vitamin D is a fat-soluble vitamin, which is mainly produced in the skin from sun exposure. To become biologically active, vitamin D undergoes two hydroxilations in the liver and kidney. There are two important forms of vitamin D (D_2 and D_3). While vitamin D_3 is produced in the body, D_2 is derived from food and supplements.

Objective: We wanted to show the value of vitamin D_3 and total vitamin D (D_2 and D_3) in the same patients.

Materials and methods: The study included 40 patients (30 women and 10 men). The values of vitamin D_3 and total vitamin D were determined by the immunoassay on analyzer COBAS e601 (Roche, USA).

Results: The study group value of vitamin D_3 < 10 nmol/L was found in 6 patients, 10-30 nmol/L in 10 patients, 30-75 nmol/L in 22 patients and > 75 nmol/L in 2 patients. Total vitamin D was determined for the same patients; the value of total vitamin D < 10 nmol/L was not detected in any patient, 10-30 nmol/L in 5 patients, 30-75 nmol/L in 23 patients and > 75 nmol/L in 11 patients.

Conclusion: Based on the results we can conclude and confirm that the method for total vitamin D determines both vitamin D_3 and vitamin D_2 because the values are higher than those from the method that determines only vitamin D_3.

P22-06

Comparison of two immunoassays for 25-OH vitamin D

Mägi M

East-Tallinn Central Hospital, Central Laboratory, Tallinn, Estonia

Corresponding author: maiga.magi@itk.ee

Background: There are several analytical methods used for measuring the concentration of 25-OH vitamin D in serum. The purpose of this study was to compare the concentrations of vitamin D using two different immunoassays: electrochemiluminescence immunoassay (ECLIA, Roche Cobas e411) and enzyme-linked immunosorbent assay (ELISA, Euroimmun AG).

Material sand methods: First the vitamin D concentrations in serums were measured with ECLIA and according to this results were divided into three groups based on vitamin D recommendations by National Osteoporosis Foundation. First group – vitamin D concentration below 25 nmol/L (50 serums), second group -concentration between 25-75 nmol/L (48 serums) and third group – concentration 75 nmol/L or higher (50 serums). All the serums of three groups were re-measured with both methods in one batch at the same day.

Results: The coefficient of determination (R^2) was 0.577 ($P < 0.001$) for the first, 0.859 ($P < 0.001$) for the second and 0.669 ($P < 0.001$) for the third group. The linear regression between methods was ELISA (y) = 1.28*ECLIA (x) + 24.66 (SE = 5.59) for the first, ELISA(y) = 0.94*ECLIA (x) + 17.73 (SE = 5.96) for the second and ELISA (y) = 0.89*ECLIA (x) + 25.46 (SE = 11.15) for the third group.

Conclusions: There was no good correlation between two methods used in this study. The correlation between two assays was best for the second group containing serums with 25-OH vitamin D concentrations between 25-75 nmol/L. Additional studies containing more methods are required to evaluate and compare the methods used for measurement of 25-OH vitamin D.

P22-07

25-hydroxi-vitamin-D levels in clinical conditions with low plasma albumin

Kovacs G (1), Horvath D (2), Viragh E (2), Kovacs L (3), Locsei Z (2), Toldy E (4)

(1) University of Pecs, Institute of Laboratory Medicine, Pecs, Hungary
(2) Markusovszky Teaching Hospital, 1st Department of Medicine, Szombathely, Hungary
(3) Markusovszky Teaching Hospital, B. Braun Avitum Dialysis Centre, Szombathely, Hungary
(4) University of Pecs, Institute of Diagnostics, Szombathely, Hungary

Corresponding author: gabor.l.kovacs@aok.pte.hu

Introduction: Majority of circulating total 25(OH) D is bound to proteins. 90% is bound to vitamin D binding protein (DBP), an alfa-2globulin (a-2-Gl)

fraction, and 10% to albumin (ALB). Nowadays the total t25(OH)D level is considered to be the most widely accepted marker of vitamin D supply in physiological states. Under pathological circumstances (e.g. in diseases with low ALB), however, the situation might be different. Our aim was to investigate the t25(OH)D concentration in clinical conditions with low ALB levels.

Materials and methods: 95 patients (48 men, 47 women; mean age: 68.2 ± 14.4 years) with low ALB (31.9 ± 5.9 g/L) were studied. 58 patients had chronic renal failure, 8 nephrosis, 17 cirrhosis and 12 malnutrition. 58 healthy adults (30 men, 28 women; mean age: 65.9±15.8 years) were in the control group. 25(OH)D, intact parathormon (PTHi), calcium, TP, ALB, DBG, a-2-Gl were measured.

Results: 90% of the patients with low ALB had vitamin D deficiency (< 50 nmol/L). Low vitamin D, however, also occurs among healthy people. Vitamin D deficiency is much more frequent in cases with low DBP (< 272 mg/L), than in those with normal DBP. There was a correlation between vitamin D and DBP. This was much stronger in the group of patients with renal failure.

Conclusion: Our results suggest that in clinical conditions with low ALB levels - especially if hypalbuminaemia is associated with low DBP and excess of a-2-Gl - t25(OH)D levels may not only depend on vitamin D supply, but also on the presence and capacity of the binding proteins.

P22-08

Intraoperative parathyroid hormon measurements in femal with parathyroid adenoma

Mayer L (1), Gaće M (1), Dobrijević S (1), Špacir-Prskalo Z (1), Janušić R (2), Ramljak V (3)

(1) Sestre Milosrdnice University Hospital, University Hospital for Tumors, Clinical Institute of Chemistry, Zagreb, Croatia
(2) Sestre Milosrdnice University Hospital, University Hospital for Tumors, Department of Head and Neck Surgery, Zagreb, Croatia
(3) Sestre Milosrdnice University Hospital, University Hospital for Tumors, Department of Cytology, Zagreb, Croatia

Corresponding author: ljiljana.mayer@kbcsm.hr

Primary hyperparathyroidism (PHPT) is disorder characterized by increased and uncontrolled parathyroid hormone secretion, cause of hyperfunction of one or more parathyroid glands. In 80-85% cases of PHPT is caused by parathyroid adenoma. Persistent hyperparathyroidism leads to altered osseous metabolism involving bone resorption and tissue changes. In rare cases, approximately in every thirteenth patient with PHPT, the bone mass is suspected of being a neoplastic lesion - brown tumor induced by primary hyperparathyroidism. The only way of PHPT correction is surgical elimination hyperactive parathyroid glands. In this article we report the first case of intraoperative parathyroid hormone measurements for primary hyperparathyroidism in Republic of Croatia. The possibility of intraoperative PTH monitoring provides an additional patient and operator safety. Intraoperative PTH becomes an exact instructor (navigator) to operator –surgeon. On the basis of decreasing value of this peptide with very short half-life time, surgeon makes immediate decision if the operation is completed or he requires further excision parathyroid glands because of hyperplasia. Guarantee of successful diagnosis, which is a prerequisite for the correct treatment, is a multidisciplinary, continuous, systematic and synchronized cooperation of whole and heterogeneous medical team, which includes clinicians, radiologists, cytologists, pathologists and medical biochemists.

P22-09

Patients with colorectal cancer have profound deficiency of vitamin D

Greplova K (1), Valik D (1), Obermannova R (2)

(1) Masaryk Memorial Cancer Institute, Department of Laboratory Medicine, Brno, Czech Republic
(2) Masaryk Memorial Cancer Institute, Department of Comprehensive Cancer Care, Brno, Czech Republic

Corresponding author: greplova@mou.cz

Background: Vitamin D plays an important role in a number of physiological functions including calcium absorption, bone metabolism, immune function, muscle function and cellular regulation. Numerous clinical studies have shown that vitamin D has significant protective effect against the development of cancer. We studied changes in 25(OH)D serum concentrations at different temperature storage conditions. Subsequently we measured 25(OH)D serum concentration in 100 healthy individuals and 281 patients with colorectal cancer.

Materials and methods: Blood samples were taken in sample tube without anticoagulant (Sarstedt, catalog number 01.1728.001). After centrifugation (1500 x g, 15˚C, 20 min), separated serum was pipetted into 3 test tubes. 25(OH)D was measured 1) immediately after serum separation 2) after 3 weeks of freezing at -80 ˚C 3) after 3 weeks of freezing at -30˚C. 25(OH)D serum concentrations were measured using Architect i2000sr (Abbott) analyzer.

Results: We didn't find any significant concentration changes of 25(OH)D in 100 samples at various temperature storage conditions. In the group of healthy individuals median of 25(OH)D concentration was 55.35 nmol/L (range within 21.7-116.4 nmol/L), yielding reference range 30-90 nmol/L (95% confidence interval). In the group of colorectal cancer patients median of 25(OH)D was 26.1 nmol/L (range within 0-84.7 nmol/L).

Conclusions: The CMIA method proved to be robust and stable for clinical determination of serum 25(OH)D levels. In colorectal cancer patients we observed profound deficiency of vitamin D.

This study was funded by European Regional Development Fund and State budget of the Czech Republic (RECAMO:CZ 1.05/2.1.00/03.0101) and by Ministry of Education, Youth and Sports (BBMRI:LM2010004).

P22-10

Vitamin D mediated inhibition of cancer: Do cytokines play a role?

Mohapatra S (1), Saxena A (1), Gandhi G (2), Singh T (3), Koner BC (1), Ray PC (1)

(1) Maulana Azad Medical College, Department of Biochemistry, New Delhi, India
(2) Maulana Azad Medical College, Department of Obstetrics and Gynecology, New Delhi, India
(3) Maulana Azad Medical College, Department of Pathology, New Delhi, India

Corresponding author: sudhesna.moh@gmail.co

Background: There exists an inverse relationship between vitamin D levels in blood and incidence of many cancers. Vitamin D suppresses pro-inflammatory Th1 cytokines (TNF-α) and promotes Th2 subtype differentiation (as marked by rise in IL-4). In ovarian cancer, the tumour microenvironment is enriched with a broad spectrum of pro-inflammatory cytokines which helps in tumour progression. Role of vitamin D in ovarian cancer has not yet been clearly defined.

Materials and methods: A case control study was conducted recruiting fifty ovarian cancer patients and fifty controls. Serum vitamin D, TNF-α and IL-4 were measured in fasting blood sample of the subjects.

Results and conclusions: Serum vitamin D levels were significantly ($P < 0.033$) lower in ovarian cancer cases [20.1 ng/mL (61.8-6.93)] as compared to controls [4.6 ng/mL (47-7.3)] which was more evident in post-menopausal group of ovarian cancer patients. TNF-α levels were significantly higher in ovarian cancer patients [cases: 12.2 pg/mL (21.0-

5.1); controls: 6.2 pg/mL (12.0-2.0); P < 0.001] and IL-4 levels were significantly lower as compared to those of controls (cases: 2.22 ± 0.51 pg/mL; controls: 2.99 ± 0.68 pg/mL; P < 0.001). Vitamin D levels were negatively correlated (R^2 = 0.092, P < 0.034) with TNF-α and positively correlated (R^2 = 0.227, P < 0.001) with IL-4. None of the ovarian cancer patients had serum vitamin D level in highest tertile of the study group. Our study provides evidence that increased Th1 cytokines release and decreased Th2 response are associated with low serum vitamin D which might be a risk factor for ovarian cancer. This indicates that supplementing vitamin D might be protective against ovarian cancer (especially in postmenopausal women) by modulating the cytokine environment.

Index of authors at the 2nd EFLM-UEMS Congress

Lecture authors

Poster authors

A

B

C

Č

Ć

D

Đ

E

F

G

H

I

J

K

L

Lj

M

N

O

P

R

S

Z

Ž